JOURNAL OF AGRICULTURAL RESEARCH

VOL. 59 WASHINGTON, D. C., SEPTEMBER 1, 1939 NO. 5

COMPOSITION OF THE VARIOUS PARTS OF THE OAT PLANT AT SUCCESSIVE STAGES OF GROWTH, WITH SPECIAL REFERENCE TO THE FORMATION OF LIGNIN[1]

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INTRODUCTION

The present paper is one of a series (*9*, *36*, *38*)[3] from this Bureau dealing with the chemical composition of certain crop plants at different stages of growth and development. The object of these studies was to determine the nature and percentage of the more important components of the plant, both structural and nonstructural, as well as to ascertain their possible interrelationships, with a view to learning more about the formation of lignin by the growing plant and the mechanism involved in the process of lignification.

This paper reports a study of the composition of several parts of the oat plant at different stages of development. Samples were taken at weekly intervals, beginning 1 week after the plants emerged and continuing until they became mature and senescent. Inasmuch as growth is a dynamic process, results giving only the percentage composition of a plant at different stages of development may be deceptive and misleading, for an increase in the percentage of one component may not necessarily indicate an actual increase in the absolute quantity of this constituent, but may be due rather to a diminution or translocation of some other component or components. Accordingly, in this investigation not only were the percentages of the more important constituents determined, but their absolute quantities were also ascertained at successive stages of development of different parts of the oat plant. In view of the fact that the primary object of this investigation was to study the development of lignin in the plant, no chemical examination of the grain at different stages of development was undertaken.

REVIEW OF LITERATURE

A review of the literature relative to the composition of certain plants at different stages of growth was presented in a previous paper (*38*). In this paper, a review will be given of the more recent literature on the subject, together with a résumé of the older publications that are of particular interest in connection with the results of the present investigation.

[1] Received for publication May 6, 1939. Investigation conducted in cooperation with the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Idaho Agricultural Experiment Station. A résumé of this paper was presented on April 6, 1939, at the ninety-seventh meeting of the American Chemical Society, held at Baltimore, Md.

[2] The writers express their thanks to T. R. Stanton, of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, for many helpful suggestions made in the course of this investigation.

[3] Italic numbers in parentheses refer to Literature Cited, p. 363.

About 90 years ago the American agricultural chemist Norton (*32*), working in the laboratory of the Agricultural Chemistry Association of Edinburgh, Scotland, made an extensive study of the composition of the oat plant at different stages of growth. He determined the percentage of ash as well as the chemical composition of the ash in the clums, leaves, knots (culm nodes), chaff, and grain. He found that the percentage of ash in the leaf and in the chaff increased steadily as the plants grew older, while in the stalks, after an initial increase, it decreased as the plants matured. The percentage of ash in the knots was found to be greater than that in the entire stalk. He also reported that there was a difference in the percentage of ash yielded by different parts of the same stalk. There was not, however, a regular gradation in the percentage of ash from the top of the stalk downward.

Collins and Spiller (*7*) reported that green oat straw may contain approximately 6 percent of sugar, mostly invert sugar, while fully mature straw contains practically no sugar.

Rogozinski and Starzewska (*41*) determined the percentage of ash, crude fiber, pentosans,and methoxyl at successive stages of growth of the oat plant. Lignin was not determined directly, but was calculated from the percentage of methoxyl found, the assumption being that the percentage of methoxyl in lignin from cereal straw is 13.50 and that lignin is the only methoxyl-containing substance in the oat plant. It is now known, however, that this assumption is not true, so that their lignin values cannot be accepted without reserve. Their results showed that the percentage of crude fiber, pentosans, methoxyl, and lignin increased as the plant grew older, while the ash decreased.

Fagan and Watkin (*13*) found that the crude protein in the leaves and culms decreased rapidly with the increase in the age of the oat plants. The percentage of ash in the culms at first increased, and then decreased somewhat as the plants matured. The percentage of ash in the leaves decreased steadily with the increase in the age of the plants.

Wagner (*47*) reported that during the later stages of development of the oat plant there was a constant decrease in the weight of the leaves and culms. This, however, did not apply to the top growth of the plants as a whole.

Phillips and Goss (*38*), in studying the composition of the leaves and stalks of the barley plant at successive stages of growth, found that after an initial increase the percentage of ash and nitrogen declined steadily as the plant matured. The percentage of methoxyl in the original and in the extracted plant materials increased with the age of the plant. The percentage of alcohol-benzene and cold- and hot-water extractives declined, though not regularly, as the plant became older. The percentage of uronic acids increased somewhat during the early development of the plant, and then declined as the plant matured. The percentage of the furfural-yielding components, as well as the percentage of pentoses calculated as pentosans, increased as the plant matured. The percentage of cellulose, Cross and Bevan cellulose, the furfural-yielding fraction of Cross and Bevan cellulose, and lignin, as well as the percentage of methoxyl in the lignin, also increased regularly as the plants developed and matured. No direct evidence was obtained that the barley plant synthesizes lignin from cellulose, pentoses, or pentosans.

Norman (*29*) and Norman and Richardson (*30*), who studied the composition of ryegrass (western wolths, *Lolium multiflorum*) at different stages of development, found that the percentage of cellulose, xylan in the Cross and Bevan cellulose, and lignin increased progressively with the age of the plant. No direct determination of the polyuronide hemicelluloses was made, but from the percentage of furfural found it was concluded that they did not increase regularly and were lower in the mature grass than in the young grass. A water-soluble fructosan was found in considerable quantity in the younger plants. The maximum percentage of the fructosan was attained at about the time of full emergence of the head, and it then decreased as the plant matured.

Many suggestions are found in the literature concerning the nature of the parent substance and the possible mechanism involved in the synthesis of lignin by the plant. Most of these suggestions are either purely speculative or are based on indirect or fragmentary evidence.

Klason (*20*) believes that lignin is formed by the plant from pentoses or methyl pentoses and that coniferyl alcohol (or coniferyl aldehyde) is an intermediate product formed in the process. This view is also shared by Von Euler (*12*).

Rassow and Zschenderlein (*40*) point out that plant materials with a high content of lignin have a low content of pentosans, and vice versa. They suggest, therefore, that lignin, at least in part, is built up by the plant from pentosans.

According to Odén (*33*), pentoses are first converted into methyl pentoses, and these are then condensed to form benzofurane derivatives from which lignin is synthesized.

Green (*17*), Cross, Bevan, and Beadle (*8, pp. 177–181*), König and Rump (*21*), and Fuchs (*14, 15*) have suggested that lignin is formed by the plant from cellulose.

Schrauth (*45*) and Jonas (*19*) believe that soluble carbohydrates, such as pentoses and hexoses, particularly glucose, are utilized by the plant in the formation of lignin. Casparis (*6*) believes that the carbohydrates of the cell wall are the precursors of lignin.

According to an older view of Wislicenus (*48*), lignification is a process whereby the sum total of the dissolved hydrosols in the cambial sap is deposited upon the cellulose fiber. In a more recent publication (*49*), however, he suggests that while glucose is utilized by the plant in the formation of cellulose, fructose is used similarly in the synthesis of lignin.

The possibility of a biogenetic relationship between pectin and lignin has been considered by several investigators. It has been observed by Candlin and Schryver (*5*) that pectin is found rather abundantly in young and nonlignified tissues, while plants rich in lignin are relatively free from pectin. Ehrlich (*11*) isolated from a hydropectin of flax a fraction that resembled lignin in certain respects. Accordingly, he suggested that pectin is the precursor of lignin. The evidence for this, however, is not convincing.

Buston (*4*), in his studies of the process of lignification of the rose plant, proved definitely that lignin is not produced at the expense of the pectin. While in the later stages of the development of the plant, the lignin content increased considerably, the absolute quantity of the pectin did not decrease but actually increased. He concludes,

therefore, that "lignin does not arise from pectin, but more probably from some carbohydrate related to the glucosan-xylan series with members of which series it is usually associated in the plant."

According to Zherebov (*50*), primary lignin contains no methoxyl groups, and he therefore considers that lignification is a process by which methoxyl groups are accumulated. While lignin from a fully mature annual plant contains a higher percentage of methoxyl than lignin from an immature plant, it has been repeatedly demonstrated in this laboratory that lignin from even a very young plant always contains some methoxyl.

MATERIAL AND METHODS OF ANALYSIS

The oat plants (*Avena sativa* L., Victory variety) used in this investigation were grown under irrigation at Aberdeen, Idaho. The type of soil in that locality is described as an Aberdeen silty clay loam. The oat seeds were sown on April 24, 1936, and the young plants emerged on May 6. The age of the plants was calculated from the day they emerged. The first harvest took place in the morning of May 13 and subsequent samplings were made at weekly intervals, at approximately the same time of the day. The plants were taken up by the roots, freed from soil, and counted. The roots were then cut off from the culms, freed from the mother seeds, thoroughly washed in running water, and freed as nearly as possible from adhering soil. The culms and roots were dried in a steam oven at 50° C. and then spread out in the laboratory and allowed to air-dry. During heading time (when the plants were 63 days old and at each subsequent harvest period until maturity), the heads were cut off, and these were separated into grain, chaff, and rachises and branches of the panicles. With the exception of the samples from the first two harvest periods, all analyses were made on air-dried materials. Separate samples were taken for moisture and ash determinations, and the analytical data were then calculated on the moisture-free and ash-free basis. For most of the analyses, the plant materials were ground in a Wiley mill fine enough to pass through a 60-mesh sieve. Plant material ground to pass through an 80-mesh sieve was used for the determination of the percentages of material extracted by alcohol-benzene, cold and hot water, and 1 percent of hydrochloric acid as well as for the determination of lignin.

The following determinations were made:

Moisture.—A weighed air-dry sample of the material was dried at 105° C. to constant weight, and the loss in weight was determined.

Ash.—Ash was determined by igniting a weighed sample in an electric muffle furnace at 600° C. and weighing the inorganic residue.

Nitrogen and crude protein.—All nitrogen determinations were made by the Kjeldahl-Gunning-Arnold method (*2, p. 25*). The nitrogen values were also calculated as percentages of crude protein (N×6.25).

Methoxyl in original plant material.—The percentage of methoxyl in the original unextracted plant material was determined by the method described by one of the writers in a previous publication (*35*). The methyl iodide was absorbed in pyridine.

Methoxyl in extracted plant material.—The plant material was successively extracted with a 1:2 alcohol-benzene solution, cold water, hot water, and a 1-percent hydrochloric acid solution, by the pro-

cedure hereinafter described for determining these extractives, and the percentage loss in weight due to these extractions was determined. The percentage of methoxyl in the extracted plant material was then determined by the method used for the determination of methoxyl in the original unextracted plant material, and the result was calculated on the basis of the original unextracted moisture-free material as well as on the ash-free basis. The extraction operation removed alcohol-benzene-soluble and water-soluble methoxyl-containing substances as well as any methoxyl groups occurring as methyl esters of organic acids, such, for example, as are found in the pectins. The percentage of methoxyl found in the residual extracted plant material represents essentially lignin methoxyl, together with some firmly bound methoxyl (present in etherlike combination) occurring in other components of the plant.

Alcohol-benzene extractives.—These were determined by extracting a weighed sample of the material in a Soxhlet extractor for 30 hours with a 1:2 alcohol-benzene solution, and ascertaining the loss of weight.

Cold-water extractives.—To the weighed sample of the dry material extracted with alcohol-benzene solution, distilled water was added in the proportion of 150 cc. to 1 gm. of sample, and the mixture was allowed to stand at room temperature, with frequent stirring, for 48 hours. The insoluble material was filtered off and dried at 105° C., and the loss in weight was determined. The filtrate was added to a 2-liter volumetric flask and made up to the mark with distilled water (solution A), and 1,000 cc. of this solution was transferred portionwise to a weighed porcelain dish, and evaporated to dryness on the steam bath. The residual material in the dish was ignited in an electric muffle at 600°, the weight of ash was determined, and the total ash in solution A was calculated. Two 250-cc. samples of solution A were taken for nitrogen determinations by the Kjeldahl-Gunning-Arnold method (*2, p. 25*), and the total nitrogen found was calculated as grams of crude protein (N×6.25). The loss in weight due to extraction with cold water, previously determined, minus the combined weights of ash and crude protein in solution A was calculated as percentage of the original moisture-free and ash-free material.

Hot-water extractives.—To the weighed sample of the dry residue from the cold-water extraction, distilled water was added in the proportion of 150 cc. of water to 1 gm. of sample, and the mixture was boiled under a reflux condenser for 3 hours. The insoluble material was filtered off and dried at 105° C., and the loss in weight was determined. The combined weights of ash and crude protein in the filtrate were determined as described in the preceding paragraph and deducted from the loss in weight resulting from the hot-water extraction. The result obtained was calculated as percentage of the original moisture-free plant material and also as percentage of the original moisture-free and ash-free material.

One-percent hydrochloric acid extractives.—The weighed sample of the plant material that had been successively extracted with alcohol-benzene solution, cold water, and hot water, as described above, was treated with a 1-percent hydrochloric acid solution in the proportion of 150 cc. of acid solution to 1 gm. of plant material and boiled under a reflux condenser for 3 hours. The remainder of the procedure was the same as that described under cold-water extractives.

Total extractives.—The total extractives are the sum of the percentages of the alcohol-benzene, cold-water, hot-water, and 1-percent hydrochloric acid extractives.

Uronic acid anhydrides.—The uronic acid anhydrides were determined in the unextracted material according to the procedure recommended by Dickson, Otterson, and Link (*10*), as modified slightly by Phillips, Goss, and Browne (*37*). The uronic acids are present chiefly in the pectins and in the hemicelluloses.

Total furfural.—Furfural was determined in the original unextracted plant material by the method of the Association of Official Agricultural Chemists (*2, pp. 344–345*), which was the method developed by Tollens and his coworkers, particularly Kröber (*22*). The weight of furfural corresponding to the weight of furfural-phloroglucide was obtained from Kröber's table (*2, pp. 641–643*).

Pentosans.—Pentosans represent the difference between the total furfural and the furfural derived from the uronic acids calculated as percentage of pentosans.

When uronic acids are boiled with 12-percent hydrochloric acid, as in the determination of pentosans, they are decomposed into furfural, carbon dioxide, and water. While the yield of carbon dioxide is quantitative (this fact is the basis of several methods described in the literature for the quantitative estimation of uronic acids), the yield of furfural is not. Lefèvre and Tollens (*25*) distilled glucuronic acid anhydride and derivatives of this compound with 12-percent hydrochloric acid, under the experimental conditions prescribed for the determination of pentosans, and showed that the weight of furfural phloroglucide produced amounted to one-third of the weight of glucuronic acid anhydride present. By substituting the weights of furfural taken from Kröber's table (*2, pp. 641–643*) for the weights of furfural phloroglucide obtained by Lefèvre and Tollens in 12 determinations and on 4 different products, it was calculated that their average yield of furfural amounted to 19.11 percent of the weight of glucuronic acid anhydride, or the ratio of furfural to glucuronic acid anhydride was 1 to 5.23, Norris and Resch (*31*) distilled euxanthic acid with 12-percent hydrochloric acid and found that the average yield of furfural in 8 determinations amounted to 21.48 percent of the weight of the glucuronic acid anhydride so that the ratio of furfural to glucuronic acid anhydride was 1 to 4.66. The mean value of the two ratios is 1 to 4.95. The yield of furfural from galacturonic acid anhydride is given by Norris and Resch (*31*) as 23.50 percent of the weight of the uronic anhydride so that the ratio of furfural to galacturonic acid anhydride is 1 to 4.26. Accordingly, to determine the percentage of furfural corresponding to the uronic acids present, the percentages of the latter were divided by the factor 4.60 (mean value of 4.95 and 4.26. Thus,

$$\frac{\text{percentage of uronic acid anhydride}}{4.60} = \text{percentage of furfural derived}$$

from the uronic acids. In this calculation it is assumed of course (1) that in lignified plant material both glucuronic and galacturonic acid are present and in the proportion of 1 to 1, and (2) that the yield of furfural from glucuronic acid is not affected by the presence of galacturonic acid, pentoses, hexoses, etc., and (3) that the yield of furfural from galacturonic acid is not affected by the presence of glucuronic acid, pentoses, hexoses, etc. However, it is believed that a reasonably

close approximation of the yield of furfural derived from the uronic acids is obtained by the above-described method. In the present state of knowledge of the composition of the plant cell wall, it is not possible to obtain results of greater precision and accuracy. The difference between the percentage of total furfural and the percentage of furfural derived from the uronic acids when multiplied by the factor 1.71 gives the percentage of pentosans, that is, the pentosan fraction associated with the Cross and Bevan cellulose plus that present in the polyuronide fraction.

Cross and Bevan cellulose.—The weighed sample contained in a 60-cc. sintered-glass crucible (porosity 2 to 3) was placed in a 150-cc. beaker, and a boiling 1 to 2 alcohol-benzene solution was added to within one-half inch of the top of the crucible. As the solvent drained into the beaker, more alcohol-benzene solution was added until the level of the solvent in the beaker was the same as that in the crucible. The beaker, covered with a watch glass, was then placed on the steam bath and allowed to remain there for one-half hour. The temperature of the steam bath was so regulated that the alcohol-benzene solution in the beaker was just below its boiling point. The solvent in the crucible was removed with suction, and the extraction with hot alcohol-benzene solution was repeated twice, and then was extracted with hot 95-percent ethanol. The extracted plant material in the crucible was washed with boiling water, the excess water was removed with suction, and the crucible and contents were allowed to cool to room temperature. The plant material was then alternately chlorinated and digested with a 2-percent sodium sulfite solution, then bleached with a 0.1-percent potassium permanganate solution, and finally treated with a sulfurous acid solution, as described by one of the writers in a previous paper (*35*). The bleached Cross and Bevan cellulose was removed from the crucible with the aid of a glass rod, and the cellulosic material, as well as the nearly empty crucible, was placed in an 800-cc. beaker. Three hundred cubic centimeters of boiling water was added to the beaker, and the mixture was digested on the steam bath for 2 hours. The crucible was removed from the beaker, and any adhering cellulosic material was washed into the beaker. The contents of the beaker were then added portionwise to the crucible, filtered, washed successively with hot water, 95-percent ethanol, and ether and finally dried for 4 hours at 105° C. and weighed. The percentage of ash in a portion of the Cross and Bevan cellulose was determined, and the percentage of ash-free Cross and Bevan cellulose was then calculated.

Furfural and xylan from Cross and Bevan cellulose.—This determination was made by the same method used for the quantitative estimation of the total furfural in the plant materials. The percentage of furfural was calculated on the basis of ash-free Cross and Bevan cellulose. From the percentage of furfural thus found, the percentage of xylan in the ash-free Cross and Bevan cellulose was calculated. The xylan in the Cross and Bevan cellulose was also calculated on the basis of the original moisture-free and ash-free material.

Cellulose.—The percentage of xylan in the Cross and Bevan cellulose was deducted from the percentage of Cross and Bevan cellulose, and the result was expressed as percentage of cellulose in the original

moisture-free and ash-free material. Thus if X equals the percentage of xylan in the Cross and Bevan collulose and Y the percentage of Cross and Bevan cellulose, then $Y-\frac{X\times Y}{100}$=percentage of cellulose.

Reducing and nonreducing sugars.—The sugars were extracted from the weighed sample (10 gm. plus 3 gm. of calcium carbonate), the alcohol was removed, and the sugar solution was cleared according to the method of the Association of Official Agricultural Chemists (*2, pp. 134–135*). The reducing sugars were determined by the method of Munson and Walker (*2, p. 479*), and the results were calculated as percentage of dextrose in the original moisture-free and ash-free material.

The nonreducing sugars were determined in an aliquot of the sugar solution by the method of the Association of Official Agricultural Chemists (*2, pp. 341–342*), and the results were calculated as percentage of sucrose in the original moisture-free and ash-free plant material.

Extractives for lignin determination.—The extractions with 1:2 alcohol-benzene solution, cold water, hot water, and 1-percent boiling hydrochloric acid solution were carried out as already described, except that no corrections were made for the crude protein and the soluble ash in the extract. The total percentage loss due to these extractions was calculated.

Lignin.—This was determined in the extracted plant material by the method of Goss and Phillips (*16*). The nitrogen in the lignin, determined by the Kjeldahl-Gunning-Arnold (*2, p. 25*) method, is reported in the tables as percentage of the crude lignin and also as percentage of the original moisture-free and ash-free plant material. The carbohydrates generally associated with lignin in the plant do not interfere with the determination of lignin by this method (*39*).

Methoxyl in the crude lignin.—Methoxyl was determined in a portion of the crude lignin by the method already referred to. The result was calculated as percentage of the ash-free crude lignin.

Methoxyl in pure lignin.—The percentage of methoxyl in the crude lignin was determined, and the result was calculated on the basis of ash-free and crude protein-free (N × 6.25) lignin. The term "pure lignin," as used here and in the tables, has been defined in a previous communication (*38, p. 307*).

RESULTS

The results obtained are recorded in 9 tables and illustrated graphically in 9 figures.[4] Tables 1, 2, 3, and 4 show the percentage composition of the culms, sheaths, and leaves,[5] the chaff, the rachises and branches of the panicle, and the roots, respectively, of the oat plant at different stages of growth.

[4] The data relating to the last three harvest periods of the culms, sheaths, and leaves, shown in the graphs, represent in all cases the mean values of the percentages in the lower, middle, and upper thirds of the plants.

[5] The culms, sheaths, and leaves were considered as a unit in obtaining the various data recorded in this paper.

TABLE 1.—*Composition*[1] *of culms, sheaths, and leaves of oat plants at different stages of growth*

Harvest No.	Age of plants	Ash in original plant material, A	Nitrogen in original plant material		Crude protein (N×6.25)		Methoxyl in original plant material		Methoxyl in extracted plant material[2]		Alcohol-benzene extractives		Cold-water extractives		Hot-water extractives		1-percent hydrochloric acid extractives		Total ex-extractives		Uronic acids (as anhydrides)		Total furfural yielded	
			A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
	Days	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
1	7	16.07	5.12	6.10	32.00	38.12	0.69	0.81	0.14	0.17	30.08	35.85	9.03	10.76	1.24	1.48	10.98	13.09	51.33	61.18	6.98	8.32	6.50	7.74
2	14	16.78	5.53	6.64	34.56	41.50	.67	.80	.17	.20	26.85	32.26	15.72	18.88	1.63	1.96	6.60	7.93	50.80	61.03	6.58	7.90	6.29	7.56
3	21	17.98	5.60	6.83	35.00	42.69	.82	1.00	.23	.28	21.78	26.53	9.51	11.59	1.59	1.94	10.14	12.36	43.02	52.45	7.00	8.54	7.00	8.54
4	28	16.18	6.04	7.20	37.75	45.00	.80	.95	.24	.29	20.64	24.63	7.48	8.93	1.20	1.43	12.54	14.96	41.86	49.95	7.34	8.76	7.57	9.03
5	35	14.71	5.62	6.59	35.12	41.19	.74	.86	.21	.25	15.81	18.53	10.05	11.78	1.58	1.85	11.77	13.80	39.21	45.96	6.76	7.92	7.55	8.86
6	42	15.56	4.19	4.96	26.19	31.00	.84	.99	.24	.29	20.71	24.53	11.11	13.16	1.56	1.84	13.10	15.51	46.48	55.04	5.64	6.68	8.19	9.69
7	49	14.24	2.65	3.09	16.56	19.31	1.12	1.30	.43	.50	15.32	17.86	12.61	14.71	1.49	1.74	16.99	19.81	46.41	54.12	5.12	5.98	10.82	12.62
8	56	10.35	2.13	2.38	13.31	14.88	1.56	1.73	.81	.90	13.34	14.88	12.92	14.41	1.80	2.01	20.96	23.38	49.02	54.68	4.98	5.56	11.65	13.00
9	63	13.44	1.13	1.30	7.06	8.12	2.24	2.58	1.23	1.42	9.39	10.85	8.19	9.46	1.14	1.31	23.23	26.83	41.95	48.45	4.38	5.04	14.94	17.25
10	70	7.91	.85	.92	5.31	5.75	2.56	2.78	1.43	1.55	12.05	13.08	8.91	9.68	1.13	1.23	22.35	24.27	44.44	48.26	4.32	4.70	14.54	15.78
11	77	10.80	.76	.85	4.75	5.31	2.73	3.05	1.50	1.68	13.02	14.59	8.82	9.89	1.03	1.15	22.44	25.15	45.31	50.78	4.90	5.49	12.92	14.48
12	84	11.68	.57	.64	3.56	4.00	2.84	3.21	1.55	1.75	11.28	12.77	6.94	7.86	1.98	2.24	23.01	26.06	43.21	48.93	5.22	5.90	13.38	15.15
13, lower third	91	10.49	.31	.35	1.94	2.19	3.40	3.80	1.89	2.11	7.48	8.36	6.68	7.47	1.90	2.12	25.17	28.13	41.23	46.08	5.10	5.70	14.06	15.70
13, middle third	91	9.81	.40	.44	2.50	2.75	3.10	3.43	1.81	2.01	6.64	7.36	8.01	8.88	1.21	1.34	24.43	27.09	40.29	44.67	4.82	5.34	13.98	15.50
13, upper third	91	10.67	.43	.48	2.69	3.00	2.96	3.31	1.60	1.79	11.10	12.42	6.31	7.06	1.24	1.39	25.00	27.99	43.65	48.86	4.76	5.32	14.69	16.44
14, lower third	98	10.92	.33	.37	2.06	2.31	3.18	3.57	2.26	2.54	5.85	6.56	6.63	7.44	1.03	1.16	25.07	28.15	38.58	43.31	5.08	5.70	14.67	16.46
14, middle third	98	10.20	.27	.30	1.69	1.88	3.23	3.60	2.06	2.29	1.42	1.59	7.48	8.33	1.10	1.22	27.22	30.31	37.22	41.45	5.06	5.64	14.84	16.52
14, upper third	98	10.45	.31	.35	1.94	2.19	3.11	3.47	2.06	2.30	3.60	4.02	6.60	7.38	1.27	1.42	26.89	30.03	38.36	42.85	5.10	5.70	15.34	17.13
15, lower third	105	11.08	.25	.28	1.56	1.75	3.34	3.76	2.12	2.39	2.89	3.25	6.37	7.16	1.35	1.52	25.83	29.05	36.44	40.98	5.38	6.04	14.12	15.87
15, middle third	105	10.59	.30	.34	1.88	2.12	3.16	3.52	2.10	2.35	3.24	3.63	6.24	6.98	1.18	1.32	26.99	29.18	36.75	41.11	4.94	5.54	14.29	15.98
15, upper third	105	11.07	.35	.39	2.19	2.44	2.98	3.36	1.89	2.13	3.85	4.33	6.12	6.88	1.02	1.15	26.13	29.39	37.12	41.75	4.76	5.36	15.22	17.11

For footnotes, see end of table.

TABLE 1.—*Composition of culms, sheaths, and leaves of oat plants at different stages of growth*—Continued [1]

Harvest No.	Age of plants	Pentosans [3]		Cross and Bevan cellulose		Furfural from Cross and Bevan cellulose [4]	Xylan in Cross and Bevan cellulose			Cellulose		Reducing sugars (as dextrose)		Nonreducing sugars as sucrose)		Total extractives in sample for lignin determination [5]	Lignin		Nitrogen in crude lignin			Methoxyl in ash-free lignin [8]	Ash in lignin [8]	Methoxyl in "pure" lignin
							(6)	On basis of plant material																
		A	B	A	B			A	B	A	B	A	B	A	B		A	B	A [6]	A [7]	B [7]			
	Days	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
1	7	8.51	10.14													77.15	1.56	1.86	4.34	0.23	0.27	2.42	43.04	4.63
2	14	8.31	9.99													76.65	1.78	2.14	4.24	.27	.33	2.14	45.75	4.18
3	21	9.37	11.42	21.0	25.5	10.89	16.96	3.56	4.32	17.5	21.2	0.93	1.13	1.79	2.18	73.25	1.56	1.90	4.44	.28	.34	2.81	47.77	5.98
4	28	10.22	12.19	22.1	26.3	12.47	19.41	4.29	5.10	17.8	21.2	.94	1.13	.48	.57	72.97	2.03	2.42	4.98	.32	.38	2.80	37.20	5.56
5	35	10.40	12.21	23.3	27.4	11.83	18.43	4.29	5.05	19.0	22.4	1.97	2.30	1.72	2.01	71.09	1.91	2.24	5.58	.34	.40	2.77	34.40	5.93
6	42	11.90	14.09	26.1	31.0	11.50	17.94	4.68	5.56	21.4	25.4	2.77	3.27	3.85	4.55	70.75	2.05	2.43	4.63	.31	.36	3.22	40.79	6.29
7	49	16.60	19.36	28.8	33.5	13.30	20.67	5.95	6.92	22.8	26.6	2.77	3.23	3.06	3.56	96.19	3.24	3.78	4.89	.37	.43	5.44	27.37	9.38
8	56	18.07	20.16	32.5	36.3	12.22	18.97	6.17	6.89	26.3	29.5	5.47	6.10	5.24	5.84	61.50	4.75	5.30	3.18	.24	.27	9.61	19.41	12.77
9	63	23.92	27.63	38.4	44.4	13.17	20.38	7.83	9.05	30.6	35.4					54.48	6.66	7.69	2.08	.23	.26	12.47	25.31	15.09
10	70	23.26	25.24	39.5	42.9	13.48	20.93	8.27	8.98	31.3	33.9	4.27	4.63	4.46	4.84	54.34	7.57	8.22	2.11	.21	.23	13.42	11.31	15.78
11	77	20.28	22.72	37.5	42.0	13.47	20.89	7.83	8.77	29.7	33.3	4.89	5.48	4.18	4.68	54.64	7.97	8.93	1.62	.18	.20	13.79	18.53	15.74
12	84	20.95	23.72	39.0	44.2	13.09	20.30	7.92	8.97	31.1	35.2	2.91	3.29	1.87	2.12	52.28	8.41	9.52	1.26	.16	.18	14.10	24.11	15.74
13, lower third	91	22.14	24.73	43.3	48.4	13.34	20.69	8.96	10.01	34.4	38.4	1.62	1.80	1.79	1.90	48.41	9.35	10.44	.57	.07	.08	16.87	20.65	17.67
13, middle third	91	23.11	24.52	41.5	46.0	12.76	19.78	8.21	9.10	33.3	36.9	1.76	1.95	2.88	3.19	49.28	8.95	9.92	.79	.09	.10	16.39	18.03	17.34
13, upper third	91	23.36	26.13	39.8	44.6	12.45	19.30	7.68	8.61	32.1	36.0	1.31	1.46	2.30	2.56	51.28	8.29	9.28	.84	.09	.10	15.77	20.08	16.87
14, lower third	98	23.20	26.03	44.6	50.1	13.75	21.32	9.51	10.68	35.1	39.4	.33	.36	.26	.28	47.52	8.88	9.97	.46	.06	.06	17.74	23.43	18.44
14, middle third	98	23.49	26.14	44.8	49.8	13.24	20.55	9.21	10.23	35.6	39.6	.34	.37	.36	.39	46.24	9.52	10.60	.51	.06	.07	16.77	16.09	17.43
14, upper third	98	24.33	27.17	44.6	49.7	13.31	20.63	9.20	10.25	35.4	39.5	.35	.39	.26	.29	46.70	9.27	10.35	.92	.11	.12	15.68	14.54	16.82
15, lower third	105	22.14	24.90	45.0	50.5	13.02	20.22	9.10	10.21	35.9	40.3	.33	.37	.32	.35	45.75	9.71	10.92	.69	.09	.10	16.92	20.00	17.89
15, middle third	105	22.61	25.27	43.8	49.0	12.54	19.44	8.51	9.53	35.3	39.5	.31	.34	.32	.35	45.23	10.12	11.32	.64	.08	.09	16.12	12.00	16.89
15, upper third	105	24.26	27.27	43.8	49.2	12.37	19.18	8.40	9.44	35.4	39.8	.32	.36	.19	.21	46.19	9.37	10.54	.73	.08	.10	15.64	15.77	16.54

[1] Results in column A were calculated on oven-dried (105° C.) material; those in column B were calculated on oven-dried (105° C.) and ash-free material.
[2] Results calculated on the original unextracted plant material.
[3] Corrected for furfural from uronic acids.
[4] Calculated as percentage of ash-free Cross and Bevan cellulose.
[5] Calculated as percentage of original moisture-free plant material.
[6] Calculated as percentage of crude lignin.
[7] Calculated as percentage of original plant material.
[8] Calculated as percentage of ash-free crude lignin.

TABLE 2.—*Composition* [1] *of chaff of oat plants at different stages of growth*

Harvest No.	Age of plants	Ash in original plant material, A	Nitrogen in original plant material		Crude protein (N×6.25)		Methoxyl in original plant material		Methoxyl in extracted plant material [2]		Alcohol-benzene extractives		Cold-water extractives		Hot-water extractives		1-percent hydrochloric acid extractives		Total extractives		Uronic acids (as anhydrides)		Total furfural yielded	
			A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
	Days	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
9	63	6.44	1.88	2.01	11.75	12.56	1.88	2.01	0.97	1.03	10.42	11.14	3.67	3.93	1.35	1.44	35.17	37.59	50.61	54.10	5.48	5.88	19.75	21.12
10	70	8.37	1.96	2.14	12.25	13.38	1.83	2.00	1.02	1.11	9.77	10.67	7.37	8.04	1.95	2.13	30.64	33.44	49.73	54.28	4.94	5.40	16.22	17.70
11	77	12.71	1.88	2.15	11.75	13.44	1.80	2.05	1.01	1.15	12.19	13.97	4.23	4.85	2.30	2.63	28.80	32.99	47.52	54.44	4.08	4.64	15.06	17.26
12	84	15.10	1.35	1.59	8.44	9.94	1.66	1.96	1.03	1.22	10.42	12.27	4.96	5.85	3.57	4.20	29.43	34.67	48.38	56.99	4.04	4.74	14.61	17.20
13	91	16.35	1.02	1.22	6.38	7.62	1.72	2.05	.97	1.16	8.64	10.32	5.15	6.16	6.40	7.65	28.69	34.30	48.88	58.44	4.36	5.24	14.80	17.70
14	98	18.13	.70	.86	4.38	5.38	1.53	2.23	.97	1.18	6.27	7.66	5.10	6.23	3.68	4.49	30.17	36.85	45.22	55.23	4.42	5.40	16.05	19.60
15	105	18.29	.77	.94	4.81	5.88	1.76	2.16	.96	1.17	8.36	10.23	4.82	5.90	4.28	5.24	29.07	35.58	46.53	56.95	4.24	5.20	16.14	19.76

Harvest No.	Age of plants	Pentosans [3]		Cross and Bevan cellulose		Furfural from Cross and Bevan cellulose [4]	Xylan in Cross and Bevan cellulose			Cellulose		Reducing sugars (as dextrose)		Nonreducing sugars (as sucrose)		Total extractives in sample for lignin determination [5]	Lignin		Nitrogen in crude lignin			Methoxyl in ash-free lignin [8]	Ash in lignin [6]	Methoxyl in "pure" lignin
							(4)	On basis of plant material																
		A	B	A	B			A	B	A	B	A	B	A	B		A	B	A	A [6]	A [7]	B [7]			
	Days	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
9	63	31.74	33.93	33.7	36.1	16.67	25.86	8.71	9.34	25.0	26.8	1.48	1.58	1.50	1.60	58.93	6.58	7.03	2.74	0.28	0.30	9.54	19.95	12.12
10	70	25.91	28.27	29.7	32.4	15.85	24.60	7.31	7.97	22.4	24.4	1.70	1.86	5.68	6.19	58.97	7.08	7.73	2.57	.29	.32	10.00	22.47	12.63
11	77	24.23	27.80	29.6	33.9	17.53	27.20	8.05	9.22	21.6	24.7	2.42	2.77	1.93	2.21	57.99	6.32	7.24	2.50	.36	.42	9.25	40.89	12.57
12	84	23.48	27.65	26.7	31.5	16.38	25.44	6.79	8.01	19.9	23.5	2.20	2.59	.75	.88	57.72	6.70	7.89	1.65	.26	.30	9.52	46.96	11.82
13	91	23.68	28.32	28.3	33.8	16.73	25.93	7.34	8.76	21.0	25.0	.96	1.13	1.42	1.70	59.06	6.75	8.07	1.41	.21	.26	10.26	47.10	12.31
14	98	25.80	31.51	29.7	36.2	15.04	23.31	6.92	8.44	22.8	27.8	.63	.76	.26	.31	55.63	7.10	8.67	.83	.14	.17	10.78	52.21	12.09
15	105	26.03	31.86	29.3	35.8	15.34	23.79	6.97	8.52	22.3	27.3	.54	.65	.23	.28	56.53	7.23	8.85	1.18	.20	.25	9.97	51.05	11.72

[1] Results in column A were calculated on oven-dried (105° C.) material; those in column B were calculated on oven-dried (105° C.) and ash-free material.
[2] Calculated on the original unextracted plant material.
[3] Corrected for furfural from uronic acids.
[4] Calculated as percentage of ash-free Cross and Bevan cellulose.
[5] Calculated as percentage of original moisture-free plant material.
[6] Calculated as percentage of crude lignin.
[7] Calculated as percentage of original plant material.
[8] Calculated as percentage of ash-free crude lignin.

TABLE 3.—*Composition* [1] *of the rachises and branches of the panicles of oat plants at different stages of growth*

Harvest No.	Age of plants	Ash in original plant, material, A	Nitrogen in original plant material		Crude protein (N × 6.25)		Methoxyl in original plant material		Methoxyl in extracted plant material [2]		Alcohol-benzene extractives		Cold-water extractives		Hot-water extractives		1-percent hydrochloric acid extractives		Total extractives		Uronic acids (as anhydrides)		Total furfural yielded	
			A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
	Days	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
9	63	4.83	1.65	1.73	10.31	10.81	2.22	2.33	1.23	1.29	6.39	6.71	4.93	5.17	2.24	2.35	33.48	35.18	47.04	49.41	5.46	5.74	19.49	20.48
10	70	4.86	1.38	1.45	8.62	9.06	2.41	2.54	1.41	1.48	11.70	12.30	3.28	3.44	2.00	2.10	30.20	31.74	47.18	49.58	4.56	4.80	17.91	18.83
11	77	5.95	1.38	1.47	8.62	9.19	2.41	2.56	1.39	1.47	10.16	10.80	3.73	3.96	2.69	2.86	31.28	33.26	47.86	50.88	4.36	4.60	16.00	17.02
12	84	7.32	1.12	1.21	7.00	7.56	2.55	2.76	1.44	1.56	11.39	12.29	3.10	3.35	3.31	3.58	31.10	33.55	48.90	52.77	4.08	4.40	15.46	16.68
13	91	10.67	.70	.78	4.38	4.88	2.64	2.95	1.42	1.59	9.79	10.96	5.77	6.46	1.83	2.04	30.56	34.21	47.95	53.67	4.34	4.86	17.06	19.10
14	98	8.75	.62	.68	3.88	4.25	2.86	3.13	1.56	1.71	8.57	9.39	3.58	3.92	2.13	2.33	31.45	34.47	45.73	50.11	4.30	4.70	17.74	19.44
15	105	9.63	.58	.64	3.62	4.00	2.90	3.20	1.55	1.72	10.86	12.02	4.18	4.63	1.73	1.91	29.62	32.78	46.39	51.34	4.32	4.76	17.88	19.78

Harvest No.	Age of plants	Pentosans [3]		Cross and Bevan cellulose		Furfural from Cross and Bevan cellulose [4]	Xylan in Cross and Bevan cellulose			Cellulose		Reducing sugars (as dextrose)		Nonreducing sugars (as sucrose)		Total extractives in sample for lignin determinations [5]	Lignin		Nitrogen in crude lignin [6]			Methoxyl in ash-free lignin [8]	Ash in lignin [6]	Methoxyl in "pure" lignin
							(4)	On basis of plant material																	
		A	B	A	B			A	B	A	B	A	B	A	B		A	B	A[6]	A[7]	B[7]			
	Days	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
9	63	31.29	32.88	36.4	38.3	15.97	24.79	9.02	9.49	27.4	28.8	1.08	1.13	2.20	2.30	56.22	7.62	8.01	2.42	0.24	0.25	11.18	7.51	13.37
10	70	28.93	30.42	36.9	38.8	16.08	24.95	9.21	9.68	27.7	29.1	1.21	1.27	5.27	5.53	54.78	8.04	8.45	2.15	.22	.23	12.72	8.59	14.90
11	77	25.73	27.39	33.7	35.8	17.19	26.69	8.99	9.56	24.7	26.3	1.78	1.89	2.81	2.99	55.95	7.84	8.34	2.39	.26	.28	12.21	13.08	14.73
12	84	24.91	26.88	33.6	36.2	16.83	26.14	8.78	9.46	24.8	26.8	2.37	2.55	1.79	1.92	56.36	8.29	8.94	1.95	.22	.24	12.27	14.19	14.30
13	91	27.56	30.85	35.5	39.8	15.61	24.25	8.61	9.65	26.9	30.2	.82	.91	1.81	2.02	55.96	8.01	8.97	1.20	.13	.14	14.10	17.07	15.50
14	98	28.74	31.50	41.9	45.9	16.44	25.49	10.68	11.70	31.2	34.2	.....	.....	.....	.....	52.21	8.47	9.28	.95	.10	.11	15.20	12.96	16.30
15	105	28.97	32.02	41.8	46.3	16.28	25.27	10.56	11.70	31.3	34.6	.....	.....	.....	.....	52.47	8.44	9.34	.83	.09	.10	14.66	15.53	15.62

[1] Results in column A were calculated on oven-dried (105° C.) material; those in column B were calculated on oven-dried (105° C.) and ash-free material.
[2] Results calculated on the original unextracted plant material.
[3] Corrected for furfural from uronic acids.
[4] Calculated as percentage of ash-free Cross and Bevan cellulose.
[5] Calculated as percentage of original moisture-free plant material.
[6] Calculated as percentage of crude lignin.
[7] Calculated as percentage of original plant material.
[8] Calculated as percentage of ash-free crude lignin.

TABLE 4.—*Composition* [1] *of roots of oat plants at different stages of growth*

Harvest No.	Age of plants	Ash in original plant material, A	Nitrogen in original plant material		Crude protein (N×6.25)		Methoxyl in original plant material		Methoxyl in extracted plant material [2]		Alcohol-benzene extractives		Cold-water extractives		Hot-water extractives		1-percent hydrochloric acid extractives		Total extractives		Uronic acids (as anhydrides)	
			A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
	Days	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
1	7	[4] 11.55	[4] 1.94	[4] 2.19	[4] 12.12	[4] 13.69	[4] 1.48	[4] 1.67	[4] 0.78	[4] 0.88	[4] 9.96	[4] 11.26	[4] 2.24	[4] 2.53	[4] 2.91	[4] 3.29	[4] 29.49	[4] 33.34	[4] 44.60	[4] 50.42		
2	14																					
3	21	10.99	2.38	2.67	14.88	16.69	2.11	2.37	.82	.92	9.71	10.91	5.95	6.68	2.75	3.09	26.40	29.66	44.81	50.34	5.68	6.40
4	28	15.58	1.84	2.18	11.50	13.62	1.47	1.74	.82	.97	6.12	7.24	2.76	3.27	2.85	3.35	28.95	34.29	40.66	48.15	5.88	7.00
5	35	12.29	2.00	2.28	12.50	14.25	1.54	1.76	.81	.92	7.17	8.17	5.46	6.22	2.88	3.28	26.98	30.76	42.49	48.43	5.61	6.41
6	42	12.01	1.25	1.42	7.81	8.88	1.83	2.07	1.02	1.15	4.06	4.61	4.39	4.99	2.35	2.63	29.99	34.08	40.79	46.36	5.06	5.76
7	49	13.08	.95	1.09	5.94	6.81	2.19	2.51	1.26	1.45	2.91	3.35	3.07	3.53	1.93	2.22	30.24	34.79	38.15	43.89	5.04	5.80
8	56	20.69	.64	.81	4.00	5.06	2.31	2.90	1.44	1.69	1.93	2.43	6.88	8.68	2.39	3.01	24.00	30.25	35.20	44.37	4.60	5.80
9	63	14.73					2.75	3.22	1.61	1.88	6.92	8.12	5.55	6.51	2.22	2.61	24.94	29.25	39.63	46.49	4.64	5.44
10	70	11.35	.50	.54	3.12	3.50	3.20	3.61	1.92	2.16	3.95	4.46	4.82	5.43	2.08	2.34	28.56	32.21	39.41	44.44	5.06	5.70
11	77	14.78	.42	.49	2.62	3.06	3.22	3.77	1.93	2.26	5.01	5.88	4.46	5.23	2.06	2.42	26.74	31.37	38.27	44.90	4.40	5.18
12	84	10.72	.33	.37	2.06	2.31	3.57	4.00	2.05	2.29	5.22	5.84	2.13	2.38	2.05	2.30	28.84	32.30	38.24	42.82	4.84	5.40
13	91	8.96	.32	.35	2.00	2.19	3.80	4.17	2.30	2.52	1.01	1.11	2.76	3.03	2.24	2.46	31.26	34.33	37.27	40.93	4.72	5.20
14	98	5.78	.30	.32	1.88	2.00	4.06	4.31	2.50	2.65	4.12	4.37	2.68	2.84	2.10	2.23	30.84	32.74	39.74	42.18	4.78	5.08
15	105	9.17	.27	.30	1.69	1.88	4.82	5.30	2.37	2.62	6.30	6.94	1.20	1.33	1.03	1.13	30.00	33.03	38.53	42.43	4.86	5.34

For footnotes, see end of table.

TABLE 4.—*Composition of roots of oat plants at different stages of growth*—Continued

Harvest No.	Age of plants	Total furfural yielded		Pentosans [1]		Cross and Bevan cellulose		Furfural from Cross and Bevan cellulose [2]	Xylan in Cross and Bevan cellulose			Cellulose		Total extractives in sample for lignin determination [6]	Lignin		Nitrogen in crude lignin			Methoxyl in ash-free lignin [8]	Ash in lignin [7]	Methoxyl in "pure" lignin
									(3)	On basis of plant material												
		A	B	A	B	A	B			A	B	A	B		A	B	A [7]	A [8]	B [8]			
	Days	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
1	7													[4] 55.40	[4] 4.95	[5] 5.60	[4] 2.99	[4] 0.31	[4] 0.35	[4] 8.65	[4] 34.17	[4] 12.09
2	14																					
3	21	15.12	16.99	23.75	26.68									58.31	5.11	5.74	2.83	.30	.34	8.32	32.72	11.29
4	28	15.10	17.90	23.63	24.01	40.2	47.6	13.82	21.40	8.60	10.19	31.6	37.4	52.96	5.18	6.14	1.57	.24	.29	9.77	56.22	12.59
5	35	15.49	17.67	24.40	27.84	39.1	44.6	13.73	21.31	8.33	9.50	30.9	35.2	53.16	5.28	6.02	2.21	.30	.34	8.34	47.67	11.32
6	42	17.73	20.15	28.44	32.32	46.4	52.8	16.70	25.89	12.01	13.67	34.4	39.1	48.25	5.45	6.19	1.96	.26	.29	11.36	46.95	14.76
7	49	16.52	19.00	26.38	30.31	42.4	48.8	15.08	24.28	10.29	11.85	32.1	37.0	46.40	6.79	7.81	1.36	.21	.24	13.18	46.93	15.70
8	56	14.62	18.43	23.29	29.36	40.6	51.1	14.39	22.30	9.05	11.40	31.6	39.8	43.45	6.67	8.41	.76	.17	.22	14.07	66.19	16.40
9	63	13.59	15.95	21.53	25.26	39.9	46.8	13.45	20.83	8.31	9.75	31.6	37.1	47.76	7.67	8.99	.95	.17	.20	15.18	51.78	17.30
10	70	15.12	17.01	23.97	27.05	43.0	48.5	13.39	20.78	8.94	10.08	34.1	38.5	45.30	9.43	10.64	.74	.12	.14	15.79	37.62	17.05
11	77	14.32	16.81	22.84	26.81	42.4	49.8	15.36	23.85	10.11	11.88	32.3	37.9	43.28	9.31	10.92	.59	.11	.13	16.05	46.95	17.25
12	84	15.09	17.47	24.88	27.87	45.9	51.4	14.10	21.89	10.05	11.25	35.9	40.2	42.89	10.11	11.32	.51	.09	.10	14.16	41.76	14.99
13	91	16.45	18.07	25.37	28.97	47.9	52.6	15.05	23.36	11.19	12.29	36.7	40.3	42.23	11.09	12.18	.61	.10	.11	16.45	30.26	17.40
14	98	16.58	17.60	25.57	28.21	47.6	50.5	15.41	23.90	11.38	12.07	36.2	38.4	43.65	11.67	12.38	.65	.09	.10	16.60	14.27	17.44
15	105	16.10	17.73	25.72	28.33	46.0	50.7	15.50	24.07	11.07	12.20	34.9	38.5	42.64	11.39	12.54	.47	.08	.09	17.43	30.85	18.19

[1] Results in column A were calculated on oven-dried (105° C.) material; those in column B were calculated on oven-dried (105° C.) and ash-free material.
[2] Results calculated on the original unextracted plant material.
[3] Corrected for furfural from uronic acids.
[4] Samples from harvests 1 and 2 were combined and analyzed.
[5] Calculated as percentage of ash-free Cross and Bevan cellulose.
[6] Calculated as percentage of original moisture-free plant material.
[7] Calculated as percentage of crude lignin.
[8] Calculated as percentage of original plant material.
[9] Calculated as percentage of ash-free crude lignin.

ASH

The percentage of ash in the culms, sheaths, and leaves (table 1 and fig. 1) at first increased, reaching a maximum of 17.98 percent when the plants were 21 days old. Subsequently, there was a general decrease, although a rather irregular one, as the plants became older. This is in agreement with the results obtained by Norton (*32*) and by Fagan and Watkins (*13*). Similar results were obtained with wheat plants by Lawes and Gilbert (*24*), Phillips, Davidson, and Weihe (*36*), and Malhotra (*26, 27*). Norman (*28*) and Phillips and Goss (*38*) obtained similar results with barley plants. The percentage of ash in the chaff (table 2 and fig. 1) increased regularly as the plants matured.

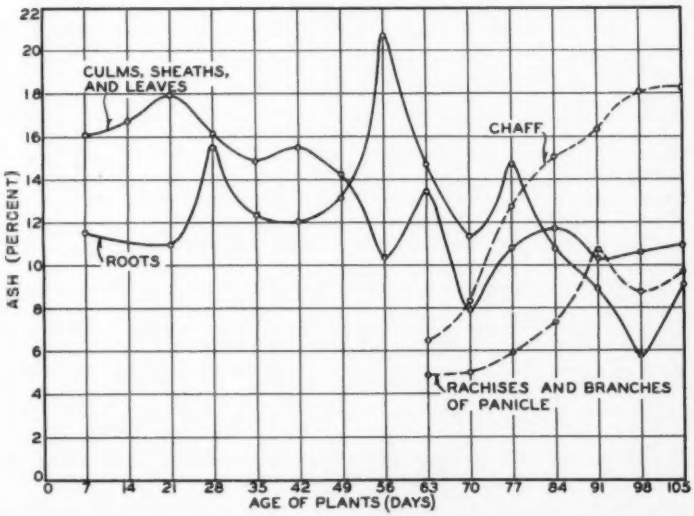

FIGURE 1.—Percentage of ash in culms, sheaths, and leaves, chaff, rachises and branches of the panicles, and roots of oat plants at successive stages of growth. (Ash values are on a moisture-free basis.)

When the plants were 63 days old it was only 6.44, whereas at maturity it had increased to 18.29 percent. The percentage of ash in the rachises and branches of the panicles (table 3 and fig. 1) increased regularly until the plants were 91 days old, after which it decreased somewhat. The variations in the percentages of ash in the roots (table 4 and fig. 1) at different stages of the development of the plants were rather irregular. In spite of the fact that the roots were washed repeatedly with water, it was not possible to free them entirely from particles of sand and other inorganic matter. Accordingly the figures on the percentage of ash in the roots are presented with some reserve.

NITROGEN AND CRUDE PROTEIN

After an early increase, the percentage of nitrogen and crude protein in the culms, sheaths, and leaves decreased generally as the plants matured (table 1 and fig. 2). The data obtained by Shaw and Wright (*46*) in their studies on the percentage of nitrogen in the corn plant at different stages of growth showed a similar tendency. Results of like character were obtained with wheat plants by Phillips, Davidson, and Weihe (*36*), and with barley plants by Phillips and Goss (*38*). In the plants from the last three harvest periods there were some differences, although not very significant ones, in the percentages of nitrogen and crude protein in the culms from the top downward. In the culms from harvests 13 (91 days) and 15 (105

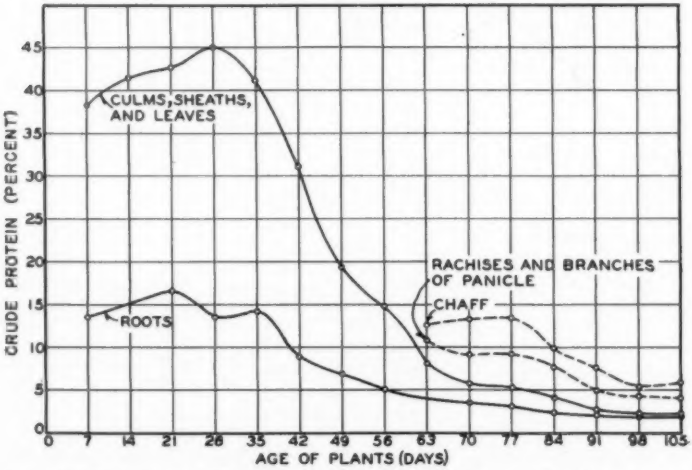

FIGURE 2.—Percentage of crude protein (on moisture-free and ash-free basis) in culms, sheaths, and leaves, chaff, rachises and branches of the panicles, and roots of oat plants at successive stages of growth.

days) the percentages of nitrogen and crude protein increased from the bottom of the stalk upward. However, this was not true of the culms from harvest 14 (98 days). The nitrogen and crude protein in the chaff and in the rachises and branches of the panicles (tables 2 and 3 and fig. 2) decreased generally, although not consistently, as the plants matured, but were considerably higher than in the culms, sheaths, and leaves from plants at the same stage of development. Thus at maturity the average percentage of crude protein (calculated on a moisture-free and ash-free basis) in the culms, sheaths, and leaves amounted to 2.10, whereas in the chaff and in the rachises and branches of the panicles the percentages of crude protein, calculated on the same basis, were 5.88 and 4.00, respectively. With the exception of the early stages of growth, the percentages of nitrogen and crude protein in the roots decreased regularly and consistently as the plants matured and became senescent (table 4 and fig. 2).

METHOXYL IN ORIGINAL PLANT MATERIAL

The percentages of methoxyl in the original plant material represent the sum of the percentages of methoxyl present either in the form of methyl esters, as in the pectins, or as methyl ethers, as in lignin. In the culms, sheaths, and leaves (table 1 and fig. 3, *A*) the percentage of

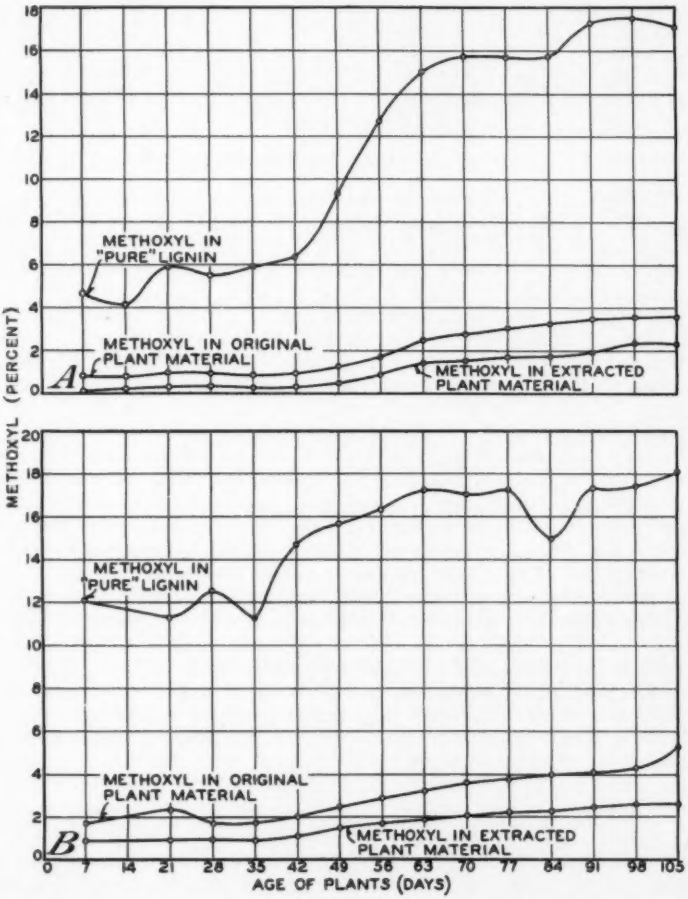

FIGURE 3.—Percentage of methoxyl in original plant material, in extracted plant material, and in "pure" lignin, in (*A*) culms, sheaths, and leaves, and (*B*) roots of oat plants at successive stages of growth. (All values are on a moisture-free and ash-free basis.)

methoxyl increased, in general, with the increase in the age of the plants until the plants were 91 days old. Subsequently, although there were some variations, the percentages on the whole were of the same general order of magnitude. The percentage of methoxyl in

the chaff (table 2) showed no appreciable change, but in the rachises and branches of the panicles (table 3) it increased regularly and consistently with the increase in the age of the plants. The percentage of methoxyl in the roots (table 4 and fig. 3, *B*) increased regularly with the increase in the age of the plants, except in the sample from the fourth harvest (28 days). The percentage of methoxyl in the roots was greater than that in any of the other parts of the oat plant. Thus at maturity, the percentage of methoxyl in the roots calculated on a moisture-free and ash-free basis was 5.30, while in the combined culms, sheaths, and leaves, in the chaff, and in the combined rachises and branches of the panicles the percentages calculated on the same basis were 3.55, 2.16, and 3.20, respectively.

METHOXYL IN EXTRACTED PLANT MATERIAL

The figures on methoxyl in the extracted plant material represent essentially methoxyl groups present as methyl ethers. The chief source, although not the only one, for these firmly bound methoxyl groups is lignin. In the clums, sheaths, and leaves (table 1 and fig. 3, *A*), the percentage of firmly bound methoxyl, calculated on the basis of the original unextracted plant materials, increased, in general, with the increase in the age of the plants. In the samples from the last three harvest periods, the percentages of methoxyl in the culms decreased generally from the bottom of the stalks upward. The percentage of firmly bound methoxyl groups in the chaff (table 2) increased slightly, and in the rachises and branches of the panicles (table 3) it increased somewhat more as the plants matured. The percentages of methoxyl in both cases, however, were lower than those in the culms, sheaths, and leaves from plants at the same stage of development. The percentage of firmly bound methoxyl in the roots (table 4 and fig. 3, *B*) increased generally with the increase in the age of the plants. The percentages were, in the main, higher than those in the culms, sheaths and leaves, chaff, and rachises and branches of the panicles taken from plants at the same stage of development. Attention is called to the fact that the percentage of lignin was higher in the roots than in other parts of the plant. This would explain the higher percentage of firmly bound methoxyl found in the roots, as an increase in the percentage of lignin would naturally cause a corresponding increase in the percentage of methoxyl in the extracted plant material.

ALCOHOL-BENZENE EXTRACTIVES

The percentage of alcohol-benzene extractives in the culms, sheaths, and leaves (table 1) decreased as the plants grew older, but the decrease was not uniform. As was pointed out in a previous communication (*38, p. 309*), an alcohol-benzene solution is not a selective solvent, and it removes a heterogeneous class of compounds, such as fatty and waxy substances, volatile oils, pigments, and various resinous complexes. This explains, at least in part, the irregular results obtained. The variations in the percentages of alcohol-benzene extractives in the chaff (table 2), in the rachises and branches of the panicles (table 3), and in the roots (table 4) were also irregular.

COLD-WATER EXTRACTIVES

After an increase in the percentage of cold-water extractives in the culms, sheaths, and leaves (table 1) from the second harvest period, the percentage decreased, although irregularly, as the plants matured. In the samples from harvests 12 to 15 the percentages ranged from about 7 to 9. In the chaff (table 2), rachises and branches of the panicles (table 3), and the roots (table 4) the percentages were irregular, and no definite conclusions can be drawn from them. The procedure used in this investigation for determining cold-water extractives gives results that represent the sum of the various substances (except water-soluble nitrogenous complexes and ash) removed by this treatment. It is not at all surprising, therefore, that the percentages do not show a uniform and regular trend.

HOT-WATER EXTRACTIVES

The percentage of hot-water extractives in the culms, sheaths, and leaves (table 1) showed but little variation during the development of the plants. The values for hot-water extractives were in every case considerably lower than those for the cold-water extractives. The values for the hot-water extractives in the rachises and branches of the panicles (table 3) showed the same tendency as those in the culms, sheaths, and leaves. In the chaff (table 2), however, the percentage of hot-water extractives increased with the increase in the age of the plants until the plants were 91 days old, after which it declined somewhat. The percentage of hot-water extractives in the roots (table 4) declined, although not regularly, as the plants grew older and matured.

ONE PERCENT HYDROCHLORIC ACID EXTRACTIVES

The percentage of 1 percent hydrochloric acid extractives in the culms, sheaths, and leaves (table 1) increased, although not in a uniform manner, as the plants grew older. The maximum percentage was reached when the plants were 98 days old. This general increase in the 1 percent hydrochloric acid extractives may be attributed to the fact that certain of the reserve carbohydrates, for example the hemicelluloses, in the culms, sheaths, and leaves generally increase as the plant grows older. In the chaff (table 2) and in the rachises and branches of the panicle (table 3) (calculated on a moisture-free and ash-free basis), there were no appreciable variations in the percentage as the plants developed and matured.

TOTAL EXTRACTIVES

The figures on total extractives represent the sum of the percentages of alcohol-benzene, cold-water, hot-water, and 1 percent hydrochloric acid extractives. In the culms, sheaths, and leaves (table 1) the percentage generally declined, although not regularly, as the plants matured. When the plants were young, approximately 61 percent of the plant substance (calculated on a moisture-free and ash-free basis) could be removed by successive extraction with alcohol-benzene solution, cold water, hot water, and 1 percent hydrochloric acid, while at maturity only about 41 percent could thus be extracted. In the roots (table 4)

the percentages of total extractives showed the same trend as that of the culms, sheaths, and leaves. However, the percentages in the chaff (table 2) and in the rachises and branches of the panicles (table 3) did not vary appreciably with the increase in the age of the plants.

The results on the total extractives of the samples used for determination of lignin, which were not corrected for the crude protein and soluble ash, showed the same trend as those discussed above.

URONIC ACIDS

Uronic acids are constituents of two substances of vegetable origin, namely, the pectins and the hemicelluloses. The uronic acid content of these two materials, however, is not the same; the pectins contain a much greater percentage of these acids than do the hemicelluloses.

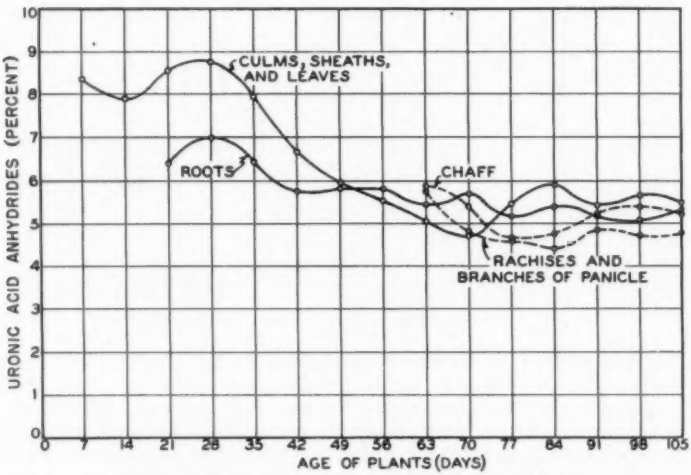

FIGURE 4.—Percentage of uronic acid anhydrides (on a moisture-free and ash-free basis) in culms, sheaths, and leaves, chaff, rachises and branches of the panicles, and roots of oat plants at successive stages of growth.

As the plants grow older, the percentage of pectin, particularly of lignified tissues, decreases, so that the percentage of uronic acids in the plant would necessarily decrease. This explains the results found in the determination of the percentage of uronic acids (calculated as anhydrides) in the culms, sheaths, and leaves (table 1 and fig. 4) of the oat plants. Similar results were obtained by Phillips and Goss (*38*) in their experiments with barley. The percentage of uronic acids (calculated as anhydrides) in the chaff (table 2 and fig. 4) and in the rachises and branches of the panicles (table 3 and fig. 4) showed the same tendency, although the decrease in percentage was not uniform and regular. The percentage of uronic acids (calculated as anhydrides) in the roots (table 4 and fig. 4) had increased slightly in the fourth harvest period (28 days), after which it decreased slowly as the plants developed and matured. In general, the percentage of uronic acids in the culms, sheaths, and leaves was greater than in the

roots taken from plants of the same age and at the same stage of development.

TOTAL FURFURAL

When a plant material is distilled with 12 percent hydrochloric acid, the total furfural obtained is derived from three principal sources, namely, (1) from the uronic acids, (2) from the pentose or pentosan fraction of the polyuronides, and (3) from the pentosan fraction forming an integral part of the Cross and Bevan cellulose. Inasmuch as some of these components increase while others decrease as the plant develops and matures and since the percentages of furfural afforded by these constituents are not the same, the percentages of total furfural recorded in the tables represent an additive effect of several factors of unequal magnitude.

The percentage of total furfural in the culms, sheaths, and leaves (table 1 and fig. 9, *A*) increased, in general, and reached a maximum

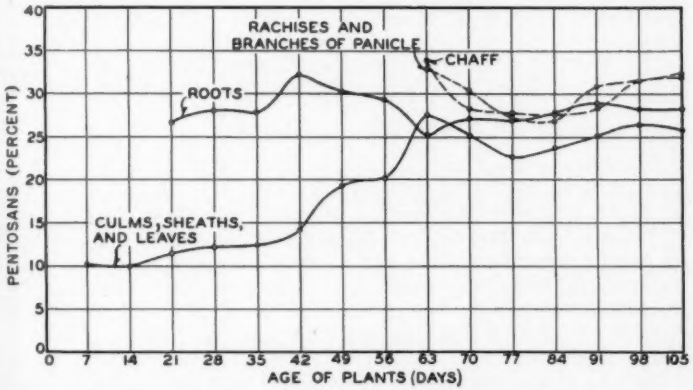

FIGURE 5.—Percentage of pentosans (on a moisture-free and ash-free basis) in culms, sheaths, and leaves, chaff, rachises and branches of the panicle, and roots of oat plants at successive stages of growth.

when the plants were 63 days old. Subsequently there was a decrease, but at or near maturity the percentage increased again. In the chaff (table 2) and in the rachises and branches of the panicles (table 3), the percentages of total furfural showed no appreciable variation (except in the samples from the ninth harvest) with the increase in the age of the plants. In the roots (table 4 and fig. 9, *B*), after an increase in the earlier stages of development of the plants, there was no great variation in the percentage of total furfural.

PENTOSANS

The percentage of pentosans was obtained by deducting from the percentage of total furfural the percentage of furfural derived from the uronic acids and calculating the difference as pentosans. These figures represent, therefore, the total pentose or pentosan units in the plant material, irrespective of whether these units occur in the gums, hemicelluloses, pectins, or in the Cross and Bevan cellulose.

Table 1 and figure 5 show that the percentage of pentosans in the culms, sheaths, and leaves increased steadily until the plants were 63 days old. Subsequently there was a small decrease in the percentage, but at maturity it had increased again. With the exception of the lower third of the sample from the thirteenth harvest (91 days), the percentages of pentosans in the samples from the last three harvests increased from the bottom of the stalk upward. In the chaff (table 2 and fig. 5) and in the rachises and branches of the panicles (table 3 and fig. 5), the highest percentage of pentosans was found in the samples from the ninth harvest (63 days). In the plants from subsequent harvests, the percentage of pentosans had decreased, but at maturity it had increased somewhat. In the roots (table 4 and fig. 5), the percentage of pentosans increased in the early stages of the development of the plants, then it decreased somewhat, and in samples taken after the ninth harvest (63 days) there was little variation.

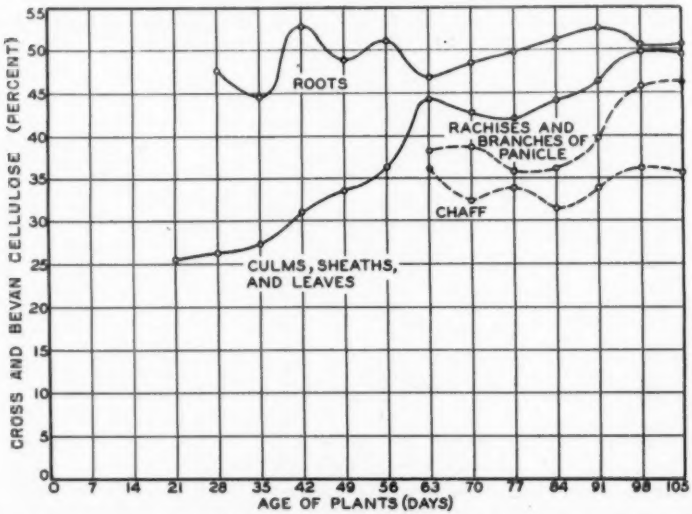

FIGURE 6.—Percentage of Cross and Bevan cellulose (on a moisture-free and ash-free basis) in culms, sheaths, and leaves, chaff, rachises and branches of the panicles, and roots of oat plants at successive stages of growth.

CROSS AND BEVAN CELLULOSE

Table 1 and figure 6 show that the percentage of Cross and Bevan cellulose in the culms, sheaths, and leaves increased from 25.5 percent (calculated on a moisture-free and ash-free basis) in the young seedlings to nearly twice this amount in the mature plants. The increase, however, was not even or regular. Similar results were obtained by Norman (*28*) and by Phillips and Goss (*38*) in their experiments with barley. In the main, in the samples from the last three harvest periods, the percentage of Cross and Bevan cellulose decreased in the culms from the bottom upward, although in some cases the decreases

were not significant. The percentage of Cross and Bevan cellulose in the chaff (table 2 and fig. 6) was irregular. From an initial percentage of 36.1 (calculated on moisture-free and ash-free basis), it had decreased to 31.5 when the plants were 84 days old, and then at maturity it had increased to about 36 again. In general, the percentage of Cross and Bevan cellulose in the chaff was found to be considerably less than in the culms, sheaths, and leaves taken from plants of the same age and at the same stage of development. The percentage of Cross and Bevan cellulose in the rachises and branches of the panicles (table 3 and fig. 6) decreased from 38.3 (calculated on a moisture-free and ash-free basis) when the plants were 63 days old to 35.8 when the plants were 77 days old. Subsequently the percentage gradually increased until at maturity it was 46.3. The percentage of Cross and Bevan cellulose in the roots (table 4 and fig. 6) increased very little as the plants grew and developed. Attention is called particularly to the fact that in the young plants the percentage of Cross and Bevan cellulose was much greater in the roots than in the culms, sheaths, and leaves. This has also been found to be true of the percentages of methoxyl in extracted plant material and lignin. The significance of this, insofar as it may throw light on the possible origin of lignin in the plant, will be discussed elsewhere in this paper.

FURFURAL AND XYLAN IN CROSS AND BEVAN CELLULOSE

The percentage of furfural yielded by the Cross and Bevan cellulose of the culms, sheaths, and leaves (table 1 and fig. 9) and, of course, also the xylan (calculated as percentage of the Cross and Bevan cellulose and as percentage of the original plant material) increased somewhat as the plants grew older. The increase, however, was neither consistent nor even. Similar results were obtained by Norman (*28*) and by Phillips and Goss (*38*) in their studies of the barley plant and by Norman (*29*) and Norman and Richardson (*30*) in their experiments on ryegrass. In the chaff (table 2) and in the rachises and branches of the panicles (table 3) the percentages of furfural (also calculated as xylan) in the Cross and Bevan cellulose did not show any pronounced variation with the increase in the age of the plants. In the roots the variations in percentage were rather irregular (table 4). In the early stages of the development of the plants, the percentage increased, then it decreased, but as the plants approached maturity there was again a small increase.

CELLULOSE

The percentage of cellulose in the culms, sheaths, and leaves (table 1 and fig. 7) increased rapidly as the plants grew older. The increase, however, was not regular. Similar results were obtained by Phillips and Goss (*38*) in their study of the composition of the barley plant at successive stages of growth. The percentage of cellulose in the chaff (table 2 and fig 7) did not vary greatly with the increase in the age of the plants. From an initial percentage of 26.8 (calculated on moisture-free and ash-free basis) when the plants were 63 days old, it had decreased to 23.5 when the plants were 84 days old, and then at maturity it had increased again to 27.3. The percentage of cellulose in the rachises and branches of the panicles (table 3 and fig. 7) increased, although not in a regular manner, as the plants grew older.

In the roots, however, the percentage of cellulose did not materially change (table 4 and fig. 7).

REDUCING AND NONREDUCING SUGARS

The percentages of reducing and nonreducing sugars (calculated as glucose and sucrose, respectively) in the culms, sheaths, and leaves (table 1) increased, in general, until heading time (ninth harvest), after which they decreased rapidly so that at maturity the percentages of both were quite negligible. Similar results were reported by Collins and Spiller (7). In the chaff (table 2) and in the rachises and branches of the panicles (table 3), the percentages of reducing and nonreducing sugars showed the same trend as those in the culms, sheaths, and leaves; that is, they increased in the earlier stages of development of the plants, after which they declined rapidly. Be-

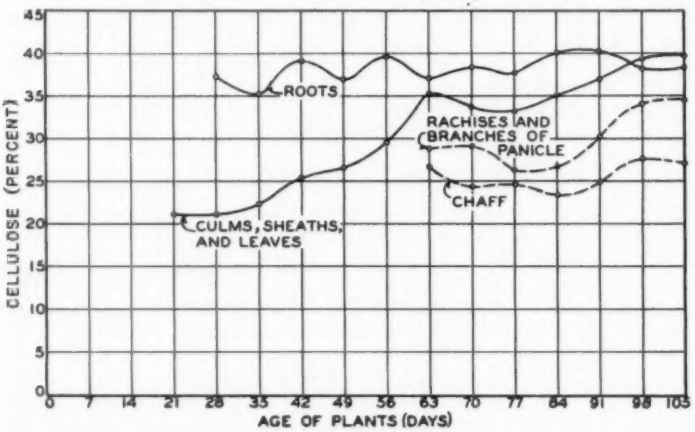

FIGURE 7.—Percentage of cellulose (on a moisture-free and ash-free basis) in culms, sheaths, and leaves, chaff, rachises and branches of the panicles, and roots of oat plants at successive stages of growth.

cause of lack of sufficient material, the percentages of reducing and nonreducing sugars in the roots were not determined.

LIGNIN, AND NITROGEN, ASH, AND METHOXYL IN THE LIGNIN

Table 1 and figure 8 show that, in general, the percentage of lignin in the culms, sheaths, and leaves increased as the plants grew older. In the first sample the percentage of lignin, calculated on the moisture-free and ash-free plant material, was 1.86, and in the last sample, when the plants were mature, it was 10.93 (mean value of the percentages of the plants from the last harvest period). Phillips, Davidson, and Weihe (*36*) found that in the wheat plant the percentage of lignin increased with the age of the plant. Similar results were obtained by Phillips and Goss (*38*) with the barley plant.

The percentage of nitrogen in the lignin (table 1) decreased as the plant grew and matured. The percentage of nitrogen in the lignin residue from the first sample was 4.34, while in the lignin from the

fully mature plant it was only 0.69 (the means value of the percentages of nitrogen in lignin from the last harvest period). Undoubtedly this was due to the fact that the percentage of nitrogen decreases as the plant grows older and matures.

The figures on the percentage of methoxyl in the ash-free lignin and in the so-called pure lignin from the culms, sheaths, and leaves (table 1 and fig. 3) show that there was a gradual increase as the plants grew older. It is rather difficult to explain this fact on the basis of Klason's (*20*) hypothesis that lignin is synthesized by the plant from coniferyl alcohol or coniferyl aldehyde. If this were the case the percentage of methoxyl in the lignin would show no such variation.

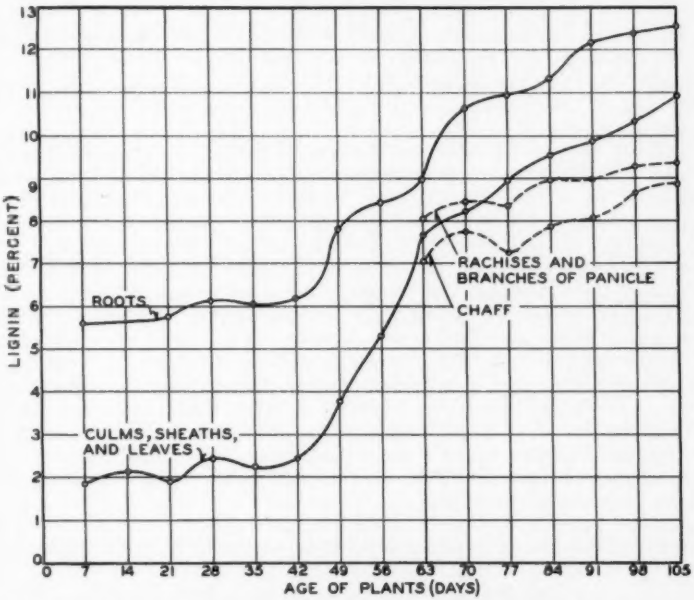

FIGURE 8.—Percentage of lignin (on a moisture-free and ash-free basis) in culms, sheaths, and leaves, chaff, rachises and branches of the panicles, and roots of oat plants at successive stages of growth.

The percentage of ash in the lignin from the culms, sheaths, and leaves (table 1) varied considerably, but in general it decreased as the plants grew older.

The percentage of lignin and the percentages of nitrogen and methoxyl in the lignin in the chaff and in the rachises and branches of the panicles are given in tables 2 and 3, respectively. The percentages of lignin are shown graphically in figure 8. In general, the percentages show the same trend as those of the culms, sheaths, and leaves. The percentages of ash in the lignin, however, differ markedly from those recorded in table 1, that is, they increase as the plant grows older. This is particularly striking in the chaff (table 2), in which the percentage of ash in the lignin at maturity was more than 50.

The percentages of lignin in the roots, as well as the percentages of nitrogen, methoxyl, and ash in the lignin, are given in table 4. The percentages of lignin and methoxyl in the lignin of the roots are shown graphically in figures 8 and 3 *B*, respectively. These constituents in the roots show the same general trend as those in the culms, sheaths, and leaves, that is, the percentages of lignin and methoxyl in the lignin increase, while the percentage of nitrogen in the lignin decreases as the plants grow older. The figures on the percentages of ash in the lignin from the roots are rather erratic because, as already explained, it was not possible to free the roots entirely from inorganic material.

PROTEIN EXTRACTED BY HOT AND COLD WATER

Table 5 gives the percentages of crude protein in the cold- and hot-water extracts (expressed also as the percentage of the total crude protein) in the culms, sheaths, and leaves, the chaff, the rachises and branches of the panicles, and the roots, respectively, at successive stages of growth.

Table 5 shows that the percentages of protein extracted from the culms, sheaths, and leaves and the roots by both cold and hot water water followed the same general trend as those of the total crude protein, that is, after an initial increase they decreased as the plant grew older. This decrease, however, was not always regular. The data on the rachises and branches of the panicles show no initial increase in the percentage of crude protein extracted by cold water, but the percentage extracted by hot water follows the same trend as that extracted from the culms, sheaths, and leaves and the roots. With few exceptions, the protein extracted by the cold water, expressed as percentage of the plant material and as percentages of the total crude protein, was considerably greater than that removed by the hot water. The percentages of protein extracted from the chaff by the cold and hot water were rather irregular. In some instances, a greater percentage of protein was extracted by the cold water, while in others this was reversed.

TABLE 5.—*Crude protein in cold- and hot-water extracts of the various parts of oat plants at successive stages of growth*

[Calculated on basis of moisture-free and ash-free plant material]

CULMS, SHEATHS, AND LEAVES

Harvest No.	Age of plants	Total crude protein	Crude protein extracted by—			
			Cold water		Hot water	
	Days	*Percent*	*Percent*	*Percent of total*	*Percent*	*Percent of total*
1	7	38.12	2.12	5.56	2.37	6.22
2	14	41.50	2.19	5.28	2.99	7.20
3	21	42.69	5.29	12.39	2.48	5.81
4	28	45.00	10.56	23.47	1.94	4.31
5	35	41.19	8.62	20.93	2.12	5.15
6	42	31.00	3.86	12.45	1.60	5.16
7	49	19.31	2.86	14.81	1.23	6.37
8	56	14.88	2.10	14.11	.68	4.57
9	63	8.12	1.40	17.24	.60	7.39
10	70	5.75	.54	9.39	.54	9.39
11	77	5.31	.43	8.10	.47	8.85
12	84	4.00	.40	10.00	.31	7.75
13, lower third	91	2.19	.32	14.61	.16	7.30
13, middle third	91	2.75	.36	13.09	.18	6.54

TABLE 5.—*Crude protein in cold- and hot-water extracts of the various parts of oat plants at successive stages of growth*—Continued

CULMS, SHEATHS, AND LEAVES—Continued

Harvest No.	Age of plants	Total crude protein	Crude protein extracted by—			
			Cold water		Hot water	
	Days	*Percent*	*Percent*	*Percent of total*	*Percent*	*Percent of total*
13, upper third	91	3.00	.16	5.33	.21	7.00
14, lower third	98	2.31	.26	11.26	.21	9.09
14, middle third	98	1.88	.23	12.23	.23	12.23
14, upper third	98	2.19	.22	10.04	.22	10.04
15, lower third	105	1.75	.22	12.57	.22	12.57
15, middle third	105	2.12	.25	11.79	.20	9.43
15, upper third	105	2.44	.37	15.16	.21	8.61
CHAFF						
9	63	12.56	1.29	10.27	0.71	5.65
10	70	13.38	1.05	7.85	1.05	7.85
11	77	13.44	.64	4.76	.77	5.73
12	84	9.94	.62	6.24	.74	7.44
13	91	7.62	.35	4.59	.62	8.14
14	98	5.38	.45	8.36	.50	9.29
15	105	5.88	.50	8.50	.50	8.50
RACHISES AND BRANCHES OF THE PANICLES						
9	63	10.81	2.16	19.98	0.63	5.83
10	70	9.06	1.69	18.65	.64	7.06
11	77	9.19	.59	6.42	.59	6.42
12	84	7.56	.48	6.35	.44	5.82
13	91	4.88	.31	6.35	.31	6.35
14	98	4.25	.28	6.59	.24	5.65
15	105	4.00	.32	8.00	.27	6.75
ROOTS						
1[1]	7	13.69	0.96	7.01	0.82	5.99
2[1]	14					
3	21	16.69	2.77	16.60	1.17	7.01
4	28	13.62	2.66	19.53	1.03	7.56
5	35	14.25	1.77	12.42	1.00	7.02
6	42	8.88	.99	11.15	.66	7.43
7	49	6.81	.89	13.07	.63	9.25
8	56	5.06	.58	11.46	.48	9.49
9	63	7.81	1.23	15.75	.50	6.40
10	70	3.50	.52	14.86	.36	10.28
11	77	3.06	.56	18.30	.22	7.19
12	84	2.31	.19	8.22	.19	8.22
13	91	2.19	.26	11.87	.13	5.94
14	98	2.00	.18	9.00	.12	6.00
15	105	1.88	.29	15.42	.17	9.04

[1] Samples from harvests 1 and 2 were combined and analyzed.

FURFURAL YIELDED BY SEVERAL COMPONENTS OF THE OAT PLANT

Table 6 shows the percentages of furfural yielded by several components of the culms, sheaths, and leaves, the chaff, the rachises and branches of the panicles, and the roots, respectively, of the oat plants at successive stages of growth. The figures in the third column of the table are taken from tables 1–4, respectively, and are inserted here for the sake of completeness and comparison. The figures in the fourth column were obtained by dividing by 4.60 the percentages of uronic acid anhydrides (ash-free basis) recorded in tables 1–4. The figures in the sixth column were obtained by multiplying the percentage of Cross and Bevan cellulose (ash-free basis) by the percentage of furfural in the Cross and Bevan cellulose (using the corresponding data

in tables 1–4), and dividing the result by 100. The figures in the eighth column represent the difference between the percentage of total furfural (third column) and the sum of the percentage of furfural from the uronic acid anhydrides and that from the pentose fraction of Cross and Bevan cellulose (fourth and sixth columns, respectively). The

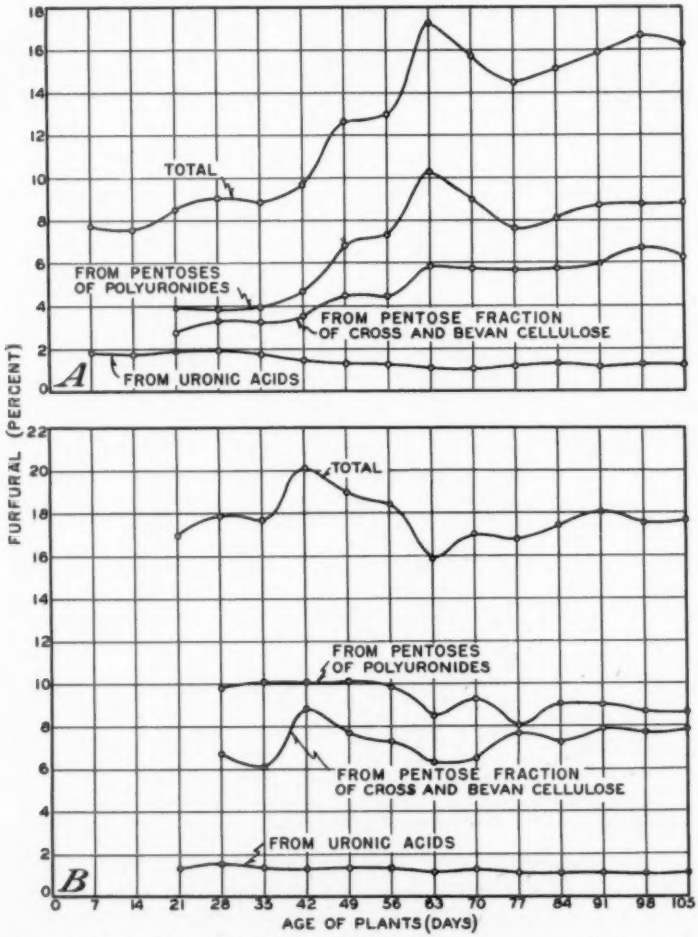
FURFURAL (PERCENT)
TOTAL
FROM PENTOSES OF POLYURONIDES
FROM PENTOSE FRACTION OF CROSS AND BEVAN CELLULOSE
FROM URONIC ACIDS
A
B
AGE OF PLANTS (DAYS)
0 7 14 21 28 35 42 49 56 63 70 77 84 91 98 105

FIGURE 9.—Percentage of furfural yielded by several components of (*A*) the culms, sheaths, and leaves and (*B*) the roots of oat plants at successive stages of growth. (All values are on a moisture-free and ash-free basis.)

figures in the last column were obtained in each case by multiplying the percentage of furfural from the pentoses of the polyuronides (eighth column) by the factor 1.736. The data recorded in table 6 on the culms, sheaths, and leaves and the roots are shown graphically in figure 9.

TABLE 6.—*Furfural yielded by several components of the various parts of the oat plant at successive stages of growth*

[Calculated on basis of moisture-free and ash-free plant material]

CULMS, SHEATHS, AND LEAVES

Harvest No.	Age of plants	Total furfural	Furfural from—						
			Uronic acids		Pentose of Cross and Bevan cellulose		Pentoses of polyuronides		Pentoses of polyuronides calculated as xylose
	Days	*Percent*	*Percent*	*Percent of total*	*Percent*	*Percent of total*	*Percent*	*Percent of total*	*Percent*
1	7	7.74	1.81	23.39					
2	14	7.56	1.72	22.75					
3	21	8.54	1.86	21.78	2.78	32.55	3.90	45.67	6.77
4	28	9.03	1.90	21.04	3.28	36.32	3.85	42.64	6.68
5	35	8.86	1.72	19.41	3.24	36.57	3.90	44.02	6.77
6	42	9.69	1.45	14.96	3.56	36.74	4.68	48.30	8.12
7	49	12.62	1.30	10.30	4.46	35.34	6.86	54.36	11.91
8	56	13.00	1.21	9.31	4.44	34.15	7.35	56.54	12.76
9	63	17.25	1.09	6.32	5.85	33.91	10.31	59.77	17.90
10	70	15.78	1.02	6.46	5.78	36.63	8.98	56.91	15.59
11	77	14.48	1.19	8.22	5.66	39.09	7.63	52.69	13.25
12	84	15.15	1.28	8.45	5.79	38.22	8.08	53.33	14.03
13, lower third	91	15.70	1.24	7.90	6.46	41.15	8.00	50.95	13.89
13, middle third	91	15.50	1.16	7.48	5.87	37.87	8.47	54.65	14.70
13, upper third	91	16.44	1.16	7.06	5.55	33.76	9.73	59.18	16.89
14, lower third	98	16.46	1.24	7.53	6.89	41.86	8.33	50.61	14.46
14, middle third	98	16.52	1.23	7.45	6.59	39.89	8.70	52.66	15.10
14, upper third	98	17.13	1.24	7.24	6.62	38.65	9.27	54.11	16.09
15, lower third	105	15.87	1.31	8.25	6.58	41.47	7.98	50.28	13.85
15, middle third	105	15.98	1.20	7.51	6.14	38.42	8.64	54.07	15.00
15, upper third	105	17.11	1.16	6.78	6.09	35.59	9.86	57.63	17.12
CHAFF									
9	63	21.12	1.28	6.06	6.02	28.50	13.82	65.44	23.99
10	70	17.70	1.17	6.61	5.14	29.04	11.39	64.35	19.77
11	77	17.26	1.00	5.79	5.94	34.42	10.32	59.79	17.92
12	84	17.20	1.03	5.99	5.16	30.00	11.01	64.01	19.11
13	91	17.70	1.14	6.44	5.65	31.92	10.91	61.64	18.94
14	98	19.60	1.17	5.97	5.44	27.76	12.99	66.27	22.55
15	105	19.76	1.13	5.72	5.49	27.78	13.14	66.50	22.81
RACHISES AND BRANCHES OF THE PANICLES									
9	63	20.48	1.25	6.10	6.12	29.88	13.11	64.02	22.76
10	70	18.83	1.04	5.52	6.24	33.14	11.55	61.34	20.05
11	77	17.02	1.00	5.88	6.15	36.13	9.87	57.99	17.13
12	84	16.68	.96	5.76	6.09	36.51	9.63	57.73	16.72
13	91	19.10	1.06	5.55	6.21	32.51	11.83	61.94	20.54
14	98	19.44	1.02	5.25	7.55	38.84	10.87	55.91	18.87
15	105	19.78	1.03	5.21	7.54	38.12	11.21	56.67	19.46
ROOTS									
1	7								
2	14								
3	21	16.99	1.39	8.18					
4	28	17.90	1.52	8.49	6.58	36.76	9.80	54.75	17.01
5	35	17.67	1.39	7.87	6.12	34.63	10.16	57.50	17.64
6	42	20.15	1.25	6.20	8.82	43.77	10.08	50.03	17.50
7	49	19.00	1.26	6.63	7.65	40.26	10.09	53.11	17.52
8	56	18.43	1.26	6.84	7.35	39.88	9.82	53.28	17.05
9	63	15.95	1.18	7.40	6.29	39.44	8.48	53.16	14.72
10	70	17.06	1.24	7.27	6.49	38.04	9.33	54.69	16.20
11	77	16.81	1.13	6.72	7.65	45.51	8.03	47.77	13.94
12	84	17.47	1.17	6.70	7.25	41.50	9.05	51.80	15.71
13	91	18.07	1.13	6.25	7.92	43.83	9.02	49.92	15.66
14	98	17.60	1.10	6.25	7.78	44.20	8.72	49.55	15.14
15	105	17.73	1.16	6.54	7.86	44.33	8.71	49.13	15.12

Table 6 and figure 9, *A*, show that after a slight variation during the early development of the plants the percentage of furfural furnished by the uronic acids of the culms, sheaths, and leaves remained fairly constant as the plants grew older and matured. When the plants were young about 23 percent of the total furfural was yielded by the uronic acids. In the later stages of the development of the plants, however, this had decreased considerably so that it ranged only from 6.78 to 8.25 percent. Similar results were obtained in previous studies with the barley plant (*38*). It will be observed from column 5, that in the samples from harvests 13, 14, and 15, the percentage of furfural from the lower, middle, and upper thirds followed in every case a regular descending order. The percentage of furfural yielded by the Cross and Bevan cellulose increased, though not regularly, as the plant grew older and matured. As in the case of the furfural from the uronic acids, the percentage of furfural decreased in the main, from the bottom of the stalk upward (harvest Nos. 13, 14, and 15). The percentage of furfural yielded by the pentoses of the polyuronides increased at first. It reached a maximum in the sample from the ninth harvest period, and subsequently declined somewhat, though not regularly, as the plant matured. The percentages of furfural from the pentoses of the polyuronides in the samples from the lower, middle, and upper thirds of harvests 13, 14, and 15 varied considerably, but here the percentage of furfural increased regularly from the lower third of the stalk upward. Throughout the development of the plant, the greatest percentage of the total furfural is furnished by the pentoses of the polyuronides. The figures in the last column show, of course, the same tendency as those recorded in the eighth column.

Table 6 shows that the percentages of furfural yielded by the uronic acids, the pentose fraction of Cross and Bevan cellulose, and the pentoses of the polyuronides of the chaff varied little with the increase in the age of the plant. Here, also, the greatest percentage of the total furfural was derived from the pentoses of the polyuronides; at maturity this amounted to more than 66 percent of the total furfural.

The percentage of furfural yielded by the uronic acids of the rachises and branches of the panicles decreased slightly, whereas the percentage of furfural from the Cross and Bevan cellulose increased, although not regularly, as the plants matured. The percentage of furfural derived from the pentoses of the polyuronides decreased somewhat as the plants matured. At maturity more than 56 percent of the total furfural was furnished by the pentoses of the polyuronides. In the plants from harvest No. 9, this amounted to more than 64 percent.

Table 6 and figure 9, *B*, show that the furfural yielded by the uronic acids from the roots decreased slightly as the plants grew older, while the percentage of furfural from the pentose fraction of Cross and Bevan cellulose was rather irregular. After an initial increase, it decreased, and finally as the plants matured it again increased somewhat. The percentage of furfural yielded by the pentoses of the polyuronides from the roots increased at first, and then decreased a little as the plants matured. In this case also, the greatest percentage of the total furfural was furnished by the pentoses of the polyuronides.

DISTRIBUTION OF FIRMLY BOUND METHOXYL GROUPS BETWEEN LIGNIN AND NONLIGNIN CONSTITUENTS

The distribution of the firmly bound methoxyl groups between the lignin and the nonlignin constituents in various parts of the oat plant at successive stages of growth is shown in table 7. The table shows that the lignin methoxyl in 100 gm. of plant material, as well as the percentage of lignin methoxyl calculated on the basis of the total firmly bound methoxyl, in the culms, sheaths, and leaves increased as the plant grew and matured. To be sure, this increase was not always regular, but the figures, in general, show an upward trend. In the fully mature plant, more than 80 percent of the total firmly bound methoxyl in the culms, sheaths, and leaves was found in the lignin. In the early stages of the development of the plant, the methoxyl not in the lignin, expressed as percentage of the total firmly bound methoxyl, was rather high, but the percentage of this decreased, in the main, as the plant grew older and matured. It has been shown by O'Dwyer (*34*), Schmidt and his coworkers (*44*), and by Hägglund and Sandelin (*18*) that in wood some of the firmly bound methoxyl groups are found also in the carbohydrate fraction. Anderson and Otis (*1*) identified methoxy glucuronic acid as one of the constituents of mesquite gum. Sands and Gary (*42*) and Sands and Nutter (*43*) showed that the hemicelluloses of mesquite wood contained the methoxyl group and that this group was associated with the fragment of hemicellulose that contained the uronic acid. Kurth and Ritter (*23*) found 3.2 percent of methoxyl in spruce "holocellulose," which was presumably freed entirely of lignin.

The lignin methoxyl in 100 gm. of plant material and the percentage of lignin methoxyl calculated on the basis of the total firmly bound methoxyl in the chaff and in the rachises and branches of the panicles did not increase very much as the plants grew and matured. At maturity, practically 90 and 85 percent, respectively, of the total firmly bound methoxyl was with the lignin.

The lignin methoxyl in 100 gm. of the roots increased almost regularly as the plants grew older. In comparing these data with those for the culms, sheaths, and leaves, it will be observed that in the roots the lignin methoxyl in 100 gm. of plant material was greater throughout the entire life of the plant. The lignin methoxyl, expressed as percentage of the total firmly bound methoxyl, ranged from 70.7 (harvest No. 3) to 87.0 (when the plants were fully mature).

WEIGHTS OF PLANTS AT DIFFERENT STAGES OF GROWTH

The weights of the culms, sheaths, and leaves, the chaff, the rachises and branches of the panicles, and the roots actually harvested and also the weights calculated on the basis of 100 plants are shown in table 8. The results present no special features. In the early stages of the development of the plants, the increase in weight in the culms, sheaths, and leaves was very rapid so that by the end of the eighth harvest period it amounted to approximately 500 times the weight at the end of the first week. The weight of the roots also increased, although to a smaller extent. In the chaff and in the rachises and branches of the panicles, the weight of dry matter increased at first, but later as maturity approached it decreased. This was presumably due to a translocation of some of the constituents from the chaff and panicles to the seed.

TABLE 7.—*Distribution of firmly bound methoxyl between lignin and nonlignin constituents of oat plants at successive stages of growth*

[Results calculated on basis of moisture-free and ash-free plant material]

Harvest No.	Age of plant	Culms, sheaths, and leaves				Chaff				Rachises and branches of panicles				Roots			
		Firmly bound methoxyl	Lignin methoxyl		Methoxyl not in lignin	Firmly bound methoxyl	Lignin methoxyl		Methoxyl not in lignin	Firmly bound methoxyl	Lignin methoxyl		Methoxyl not in lignin	Firmly bound methoxyl	Lignin methoxyl		Methoxyl not in lignin
	Days	*Grams*[1]	*Grams*[1]	*Percent of total*	*Percent of total*	*Grams*[1]	*Grams*[1]	*Percent of total*	*Percent of total*	*Grams*[1]	*Grams*[1]	*Percent of total*	*Percent of total*	*Grams*[1]	*Grams*[1]	*Percent of total*	*Percent of total*
1	7	0.17	0.09	52.9	47.1									[2] 0.88	[2] 0.68	[2] 77.3	[2] 22.7
2	14	.20	.09	45.0	55.0									.92	.65	70.7	29.3
3	21	.28	.11	39.3	60.7									.97	.77	79.4	20.6
4	28	.29	.13	44.8	55.2									.92	.68	73.9	26.1
5	35	.25	.13	52.0	48.0									1.15	.91	79.1	20.9
6	42	.29	.15	51.7	48.3									1.45	1.23	84.8	15.2
7	49	.50	.35	70.0	30.0									1.69	1.38	81.6	18.4
8	56	.90	.68	75.6	24.4									1.88	1.56	83.0	17.0
9	63	1.42	1.16	81.7	18.3	1.03	0.85	82.5	17.5	1.29	1.07	82.9	17.1	2.16	1.81	83.8	16.2
10	70	1.55	1.30	83.9	16.1	1.11	.98	88.3	11.7	1.48	1.26	85.1	14.9	2.26	1.88	83.2	16.8
11	77	1.68	1.41	83.9	16.1	1.15	.91	79.1	20.9	1.47	1.23	83.7	16.3	2.29	1.70	74.2	25.8
12	81	1.75	1.50	85.7	14.3	1.22	.93	76.2	23.8	1.56	1.28	82.1	17.9	2.52	2.12	84.1	15.9
13, lower third	91	2.11	1.84	87.2	12.8	1.16	99	85.3	14.7	1.59	1.39	87.4	12.6	2.65	2.16	81.5	18.5
13, middle third		2.01	1.72	85.6	14.4												
13, upper third		1.79	1.57	87.7	12.3												
14, lower third	98	2.54	1.84	72.4	27.6	1.18	1.05	89.0	11.0	1.71	1.51	88.3	11.7	2.62	2.28	87.0	13.0
14, middle third		2.29	1.85	80.8	19.2												
14, upper third		2.30	1.74	75.6	24.4												
15, lower third	105	2.39	1.95	81.6	18.4	1.17	1.04	88.9	11.1	1.72	1.46	84.9	15.1				
15, middle third		2.35	1.91	81.3	18.7												
15, upper third		2.13	1.74	81.7	18.3												

[1] In 100 gm. of plant material.
[2] Samples from harvests 1 and 2 were combined and analyzed.

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TABLE 8.—*Weight of plants at different stages of growth*

[Calculated on moisture-free basis]

Harvest No.	Age of plants	Height of plants	Plants harvested	Weight of plants harvested					Weight of 100 plants				
				Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Roots	Total	Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Roots	Total
	Days	*Centimeters*	*Number*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
1	7	7–10	1,020	22.34			2.32	24.66	2.19			0.23	2.42
2	14	8–17	524	31.21			3.56	34.77	5.95			.68	6.63
3	21	12–24	923	149.52			18.79	168.31	16.20			2.03	18.23
4	28	20–30	620	307.27			38.77	346.04	49.56			6.25	55.81
5	35	25–30	288	370.43			39.74	410.17	128.62			13.80	142.42
6	42	42–47	241	722.18			61.84	784.02	299.66			25.66	325.32
7	49	60–68	164	958.93			72.93	1,031.86	584.71			44.47	629.18
8	56	65–90	104	1,043.18			94.71	1,137.89	1,003.06			91.07	1,094.13
9	63	120–124	70	959.50	99.11	67.08	111.36	[1] 1,237.05	1,370.71	141.58	95.83	159.09	[1] 1,767.21
10	70	128–138	59	1,051.98	132.18	57.51	89.88	[1] 1,331.55	1,783.02	224.03	97.47	152.34	[1] 2,256.86
11	77	138–144	61	1,023.24	77.14	67.53	85.84	[1] 1,253.75	1,677.44	126.46	110.70	140.72	[1] 2,055.32
12	84	138–146	50	903.92	85.82	60.08	68.97	[1] 1,118.79	1,807.84	171.64	120.16	137.94	[1] 2,237.58
13, lower third	91	138–146	44	208.41	51.53	37.23	47.78	[1] 727.03	473.66	117.11	84.61	108.59	[1] 1,652.33
13, middle third				181.77					413.11				
13, upper third				200.31					455.25				
Total [2]				590.49					1,342.02				
14, lower third	98	138–146	37	211.28	50.60	35.14	37.43	[1] 652.34	571.02	136.76	94.97	101.16	[1] 1,763.07
14, middle third				202.18					546.43				
14, upper third				115.71					312.73				
Total [2]				529.17					1,430.18				
15, lower third	105	138–146	40	295.77	53.99	32.80	48.25	[1] 902.70	739.42	134.97	82.00	120.62	[1] 2,256.74
15, middle third				264.86					662.15				
15, upper third				207.03					517.58				
Total [2]				767.66					1,919.15				

[1] Does not include weight of the grain.
[2] Total weights of culms, sheaths, and leaves.

WEIGHTS OF DIFFERENT CONSTITUENTS AT SUCCESSIVE STAGES OF DEVELOPMENT OF THE PLANT

The weights of the more important constituents of the several parts of the oat plant at successive stages of growth are shown in table 9. In all cases, the results were calculated on the basis of 100 plants.

The data on the ash call for no special comment. There was a constant increase in the actual quantity of ash in each part during the greatest portion of the life of the plants. In the later stages of development, there was a decrease in the quantity of ash, due presumably to a translocation of the inorganic matter, in part at least, to the seed. At maturity, the ash content of certain parts of the plant again increased somewhat.

The quantity of crude protein in all parts of the oat plant except the rachises and branches of the panicle increased rapidly during the first part of the life of the plant, and then decreased steadily, although not always regularly, as the plant developed and matured. In general, the quantity of crude protein in the rachises and branches of the panicle decreased during the development of the plant.

In the total plant the content of total methoxyl as well as the methoxyl in the extracted plant material increased, although not regularly, as the plant developed and matured.

TABLE 9.—*Weight of various constituents in 100 oat plants at different stages of growth*

Harvest No.	Age of plants	Ash in—					Crude protein in—					Total methoxyl in—				
		Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Roots	Total plants	Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Roots	Total plants	Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Roots	Total plants
	Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
1	7	0.35					0.70					0.02				
2	14	1.00					2.06					.04				
3	21	2.91			0.22	3.13	5.67			0.30	5.97	.13			0.04	0.17
4	28	8.02			.97	8.99	18.71			.72	19.43	.40			.09	.49
5	35	18.92			1.70	20.62	45.17			1.72	46.89	.95			.21	1.16
6	42	46.63			3.08	49.71	78.48			2.00	80.48	2.52			.47	2.99
7	49	78.14			5.82	83.96	96.83			2.64	99.47	6.55			.97	7.52
8	56	103.82			18.84	122.66	133.51			3.64	137.15	15.65			2.10	17.75
9	63	184.22	9.12	4.63	23.43	221.40	96.77	16.64	9.88	10.64	133.93	30.70	2.66	2.13	4.37	39.86
10	70	141.04	18.75	4.74	17.20	181.82	94.68	27.44	8.40	4.75	135.27	45.64	4.10	2.35	4.87	56.96
11	77	181.16	16.07	6.59	20.80	224.62	79.68	14.86	9.54	3.69	107.77	45.79	2.28	2.67	4.53	55.27
12	84	211.16	25.92	8.80	14.79	260.67	64.36	14.49	8.41	2.84	90.10	51.34	2.85	3.06	4.92	62.17
13	91	138.77	19.15	9.03	9.73	176.68	31.77	7.47	3.71	2.17	45.12	42.39	2.01	2.23	4.13	50.76
14	98	150.76	24.79	8.31	5.85	189.71	27.06	5.99	3.68	1.90	38.63	45.53	2.50	2.72	4.11	54.86
15	105	209.35	24.69	7.90	11.06	253.00	35.32	6.49	2.97	3.73	48.51	61.04	2.38	2.38	5.81	71.61

TABLE 9.—*Weight of various constituents in 100 oat plants at different stages of growth*—Continued

Harvest No.	Age of plants	Methoxyl not removed by extraction in—					Alcohol-benzene extractives in—					Cold-water extractives in—				
		Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Roots	Total plants	Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Roots	Total plants	Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Roots	Total plants
	Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
1	7	0.003					0.66					0.20				
2	14	.01					1.60					.94				
3	21	.04			0.02	0.06	3.53			0.20	3.73	1.54			0.12	1.66
4	28	.12			.05	.17	10.23			.38	10.61	3.71			.17	3.88
5	35	.27			.11	.38	20.33			.99	21.32	12.93			.75	13.68
6	42	.72			.26	.98	62.06			1.04	63.10	33.29			1.13	34.42
7	49	2.51			.56	3.07	89.58			1.29	90.87	73.73			1.36	75.09
8	56	8.12			1.22	9.34	133.81			1.76	135.57	129.60			6.27	135.87
9	63	16.86	1.37	1.18	2.56	21.97	128.71	14.75	6.12	11.01	160.59	112.26	5.20	4.72	8.83	131.01
10	70	25.50	2.28	1.37	2.92	32.07	214.85	21.89	11.40	6.02	254.16	158.87	16.51	3.20	7.34	185.92
11	77	25.16	1.28	1.54	2.72	30.70	218.40	15.42	11.25	7.05	252.12	147.95	5.35	4.13	6.28	163.71
12	84	28.02	1.77	1.73	2.83	34.35	203.92	17.88	13.69	7.20	242.69	125.46	8.51	3.72	2.94	140.63
13	91	23.71	1.14	1.20	2.50	28.55	113.39	10.12	8.28	1.10	132.89	93.46	6.03	4.88	3.00	107.37
14	98	30.60	1.33	1.48	2.53	35.94	52.42	8.57	8.14	4.17	73.30	99.37	6.97	3.40	2.71	112.45
15	105	39.36	1.30	1.27	2.86	44.79	62.75	11.28	8.90	7.60	90.53	120.10	6.51	3.43	1.45	131.49

Harvest No.	Age of plants	Hot-water extractives in—					1-percent hydrochloric acid extracts in—					Total extractives in—				
		Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Roots	Total plants	Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Roots	Total plants	Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Roots	Total plants
	Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
1	7	0.03					0.24					1.12				
2	14	.10					.39					3.02				
3	21	.26			0.05	0.32	1.64			0.54	2.18	6.97			0.91	7.88
4	28	.59			.18	.77	6.21			1.81	8.02	20.75			2.54	23.29
5	35	2.03			.40	2.43	15.14			3.72	18.86	50.43			5.86	56.29
6	42	4.67			.60	5.27	39.26			7.70	46.96	139.28			10.47	149.75
7	49	8.71			.86	9.57	99.34			13.45	112.79	271.36			16.96	288.32
8	56	18.03			2.18	20.24	210.24			21.86	232.10	491.70			32.06	523.76
9	63	15.63	1.91	2.15	3.53	23.22	318.42	49.79	32.08	39.68	439.97	575.01	71.65	45.08	63.05	754.79
10	70	20.15	4.37	1.95	3.17	29.64	398.50	68.64	29.44	43.51	540.09	792.37	111.41	45.99	60.04	1,009.81
11	77	17.28	2.91	2.98	2.90	26.07	376.42	36.42	34.63	37.63	485.10	760.05	60.09	52.98	53.85	926.97
12	84	35.80	6.13	3.98	2.83	48.74	415.98	50.51	37.37	39.84	543.70	781.17	83.04	58.76	52.75	975.72
13	91	19.64	7.50	1.55	2.43	31.12	333.95	33.60	25.86	33.94	427.35	560.45	57.24	40.57	40.47	698.73
14	98	15.86	5.03	2.02	2.12	25.03	375.98	41.26	29.87	31.20	478.31	543.64	61.84	43.43	40.20	689.11
15	105	23.07	5.78	1.42	1.24	31.51	498.98	39.24	24.29	36.19	598.70	704.91	62.50	38.04	46.47	852.22

TABLE 9.—*Weight of various constituents in 100 oat plants at different stages of growth*—Continued

Harvest No.	Age of plants	Uronic acids (as anhydrides) in—					Total furfural yielded by—					Pentosans in—				
		Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Roots	Total plants	Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Roots	Total plants	Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Roots	Total plants
	Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
1	7	0.15					0.14					0.19				
2	14	.39					.37					.49				
3	21	1.13			0.12	1.25	1.13			0.31	1.44	1.52			0.48	2.00
4	28	3.64			.37	4.01	3.75			.94	4.69	5.06			1.48	6.54
5	35	8.69			.77	9.46	9.71			2.14	11.85	13.38			3.37	16.75
6	42	16.90			1.30	18.20	24.54			4.55	29.09	35.68			7.30	42.96
7	49	29.94			2.24	32.18	63.27			7.35	70.62	97.06			11.73	108.79
8	56	49.95			4.19	54.14	116.86			13.31	130.17	181.25			21.21	202.46
9	63	60.04	7.76	5.23	7.38	80.41	204.78	27.96	18.68	21.62	273.04	327.87	44.94	29.99	34.25	437.05
10	70	77.03	11.07	4.44	7.71	100.25	259.25	36.34	17.46	23.03	336.08	414.73	58.05	28.20	36.52	537.50
11	77	82.19	5.16	4.83	6.19	98.37	216.73	19.04	17.71	20.15	273.63	340.18	30.64	28.48	32.14	431.44
12	84	94.37	6.93	4.90	6.68	112.88	241.89	25.08	18.58	21.52	307.07	378.74	40.30	29.93	34.32	483.29
13	91	65.73	5.11	3.67	5.13	79.64	191.23	17.33	14.43	17.86	240.85	302.56	27.73	23.32	28.84	382.25
14	98	72.59	6.04	4.08	4.84	87.55	212.83	21.95	16.85	16.77	268.40	336.93	35.28	27.29	26.88	426.38
15	105	97.10	5.72	3.54	5.86	112.22	277.81	21.78	14.66	19.42	333.67	438.98	35.13	23.76	31.02	528.89

Harvest No.	Age of plants	Cross and Bevan cellulose in—					Cross and Bevan xylan [1] in—					Cellulose in—				
		Culm, sheaths, and leaves	Chaff	Rachises and branches of panicles	Roots	Total plants	Culm, sheaths, and leaves	Chaff	Rachises and branches of panicles	plants	Total plants	Culm, sheaths, and leaves	Chaff	Rachises, and branches of panicles	Roots	Total plants
	Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
1	7															
2	14															
3	21	3.40														
4	28	10.95			2.51	13.46	0.58					2.84				
5	35	29.97			5.40	35.37	2.13			0.54	2.67	8.82			1.98	10.80
6	42	78.21			11.91	90.12	5.52			1.15	6.67	24.44			4.26	28.70
7	49	168.40			18.86	187.26	14.02			3.08	17.10	64.13			8.83	72.96
8	56	325.99			36.97	362.96	34.79			4.58	39.37	133.31			14.27	147.58
9	63	526.35	47.71	34.88	63.48	672.42	61.89			8.24	70.13	263.80			28.78	292.58
10	70	704.29	66.54	35.97	65.51	872.31	107.33	12.33	8.64	13.22	141.52	419.44	35.40	26.26	50.27	531.37
11	77	629.04	37.43	37.31	59.66	763.44	147.46	16.38	8.98	13.62	186.44	558.08	50.18	27.00	51.95	687.21
12	84	705.06	45.85	40.37	63.31	854.57	131.34	10.18	9.95	14.23	165.70	498.20	27.32	27.34	45.45	598.31
13	91	557.72	33.14	30.04	52.01	672.91	143.18	11.65	10.55	13.86	179.24	562.24	34.16	29.80	49.52	675.72
14	98	638.95	40.62	39.79	48.15	767.51	111.32	8.60	7.28	12.15	139.35	453.64	24.59	22.76	39.85	540.84
15	105	849.46	39.55	34.28	55.48	978.77	133.40	9.46	10.14	11.51	164.51	505.67	31.18	29.63	36.62	603.10
							167.12	9.41	8.66	13.35	198.54	682.41	30.10	25.67	42.10	780.28

[1] Xylan fraction associated with Cross and Bevan cellulose.

TABLE 9.—*Weight of various constituents in 100 oat plants at different stages of growth*—Continued

Harvest No.	Age of plants	Reducing sugars [2] (as dextrose) in—			Nonreducing sugars [2] (as sucrose) in—			Lignin in—				
		Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Roots	Total plants
	Days	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
1	7							0.03				
2	14							.11				
3	21	0.15			0.29			.25			0.10	0.35
4	28	.47			.24			1.01			.32	1.33
5	35	2.53			2.21			2.46			.73	3.19
6	42	8.30			11.54			6.14			1.40	7.54
7	49	16.20			17.89			18.94			3.02	21.96
8	56	54.87			52.56			47.64			6.07	53.71
9	63		2.10	1.03		2.12	2.11	91.29	9.32	7.30	12.20	120.11
10	70	76.13	3.81	1.18	79.52	12.72	5.14	134.97	15.86	7.84	14.36	173.03
11	77	82.03	3.06	1.97	70.12	2.44	3.11	133.69	7.99	8.68	13.10	163.46
12	84	52.61	3.78	2.85	33.81	1.29	2.15	152.04	11.50	9.96	13.94	187.44
13	91	20.90	1.12	.69	30.85	1.66	1.53	119.00	7.90	6.78	12.04	145.72
14	98	4.83	.86		4.26	.36		131.72	9.71	8.04	11.80	161.27
15	105	6.15	.73		5.47	.31		187.31	9.76	8.92	13.74	217.73

[2] Not determined in roots.

The quantities of the various extractives in 100 plants recorded in table 9 may conveniently be considered together. In the earlier stages of the growth of the plants the weights of the extractives increased rapidly, but in the later stages they decreased. The only exception to this is the 1-percent hydrochloric acid extractives, for which the figures show, in the main, an upward trend.

In general, the uronic acids in the culms, sheaths, and leaves and the total in the plants increased as the plants grew older and matured, except in the materials from harvests 13 and 14. It will be noted throughout this table that the results from harvests 13 and 14 are, in every case, not in harmony with the rest of the data. However, the figures on the uronic acids in the chaff and the roots show at first an upward and later a downward trend. In the rachises and branches of the panicles there was a general decrease.

The content of furfural-yielding constituents in 100 plants, as well as the total pentosans, increased (with one or two exceptions) as the plants grew and developed. The greatest increase, of course, occurred in the culms, sheaths, and leaves.

The Cross and Bevan cellulose, xylan in Cross and Bevan cellulose, and cellulose increased irregularly as the plants grew and matured. The apparent decrease noted in the plants from harvests 13 and 14 is believed to be due to experimental error.

The data on the reducing and nonreducing sugars are incomplete because there was not enough material for analysis. The results presented on the content of these sugars in the culms, sheaths, and leaves, chaff, and rachises and branches of the panicles indicate that there was a rapid increase in the quantities of these sugars in the early stages of the development of the plants. As the plant grew older and matured, there was a rapid decline in the content of reducing and nonreducing sugars. No doubt to a considerable extent this was due to a translocation of the sugars from the culms and leaves to the seed, where they were stored in the form of starch.

The data on the lignin show that this constituent increased throughout the life of the plants. The data on the weight of lignin at different stages of the development of the plants when taken in conjunction with the data on cellulose are interesting. It will be observed that the actual quantities of both these components increased as the plants grew older, and therefore there is no direct evidence that the lignin increased at the expense of the cellulose.

DISCUSSION

Cross, Bevan, and Beadle (*8, p. 179*) consider that lignification is a process whereby there is a continuous modification of the cellulose, which is ultimately converted into lignin. In support of this hypothesis they state that "lignocelluloses in the first year of growth contain 70–80 p.ct. cellulose, the woods, on the other hand, 50–60 p.ct." As has already been pointed out elsewhere in this paper, results on percentage composition may be misleading in that they indicate the relative amount of each component but not the absolute quantity. A decrease in the percentage of cellulose does not necessarily mean that there was an actual disappearance of this substance, but rather that some of the other components increased at a much more rapid rate. The absolute quantity of cellulose present may have remained the

same or even may have increased somewhat. The results on the percentages and on the absolute quantities of lignin and cellulose obtained in this investigation show definitely that both these constituents increased as the plant developed and grew to maturity. There was no indication that the plant synthesized lignin at the expense of the cellulose. Moreover, considering the structure of cellulose and its function in the vegetable kingdom, it does not seem reasonable to suppose that it is used by the plant as an intermediate or as a starting material for the synthesis of other substances. There is every indication that cellulose is an end product, produced by the plant presumably from glucose, and is not utilized by it as the starting material for phytochemical syntheses. Moreover, the presence of a plant enzyme capable of bringing about a degradation of cellulose in vivo has never been demonstrated.

The theory that pectin is the parent substance utilized by the plant in the synthesis of lignin has a certain element of plausibility. Although no direct determination of pectin was made, the data on the content of uronic acids may give, indirectly, an indication of the quantity of this complex present at various stages of the development of the plant. It is realized, of course, that uronic acids are also present in the polyuronide hemicelluloses. However, while it is generally agreed that the percentage of uronic acid (as anhydride) in the pectins is about 70, it is only about one-third this percentage in the polyuronide hemicelluloses. Any appreciable loss of pectin caused by its conversion into lignin would necessarily reduce the uronic-acid content of the plant material, unless it be assumed that the synthesis of the polyuronide hemicelluloses is at once stimulated at such an accelerated rate as to compensate for the loss of pectin. While this is possible, the data obtained in this investigation definitely do not support this hypothesis. Attention is called again to the results of Buston (*4*) on the lignification of a rose shoot. Although there was a rapid increase in the lignin content, the absolute quantity of pectin did not decrease but rather increased somewhat. All in all, it may be stated that the claim that the plant synthesizes lignin from pectin is not supported by experimental results and seems, in fact, quite improbable.

The results obtained in this investigation do not support the contention of Rassow and Zschenderlein (*40*) that lignin is synthesized by the plant from pentosans. It will be observed (table 9) that the absolute quantities of both pentosans and lignin increased as the plants grew older and matured. There was no evidence that one was synthesized at the expense of the other.

If we agree that either sucrose or glucose is the first product of phytochemical synthesis, it is believed that the plant builds up lignin directly from either one of these sugars. Whether fructose alone is used in this synthesis, as claimed by Wislicenus (*49*), or whether both sugars are utilized for this purpose is, of course, not known. It may not be out of place to emphasize, however, that lignification is not, as is sometimes assumed, a senescent change. Lignin is produced even in the very earliest stages of the development of the plant. This is clear from the results obtained in this and in previous investigations (*36*, *38*). Among the first steps in the synthesis of lignin is the production of a substance or substances having firmly bound (in etherlike combination) methoxyl groups. As was

pointed out by Browne and Phillips (*3*), these may be formed in the splitting up of carbohydrates, by a process of hydrolysis, oxidation, reduction, and dehydration and not necessarily directly from formaldehyde by a process of methylation. The presence (table 7) of a large quantity of substances, presumably carbohydrates, with firmly bound methoxyl groups, which gradually decrease with the increase in the content of lignin, lends support to this hypothesis. Whether these methoxyl-containing substances are then converted into methoxyl derivatives of the pentoses by a process of oxidation and decarboxylation (via the uronic acid stage), and these in turn are converted further into lignin, is at present not known. It appears quite certain, however, that coniferyl alcohol (or aldehyde) is not one of the intermediate products in the phytochemical synthesis of lignin. The results of this and previous investigations (*36*, *38*) definitely do not support Klason's (*20*) hypothesis that the plant synthesizes lignin from coniferyl alcohol (or aldehyde) by a process of condensation or polymerization. If this were true one would expect the percentage of methoxyl in the lignin to remain substantially constant. The results of this investigation, however, show very conclusively that the percentage of methoxyl in the lignin is a variable quantity and that it increases markedly as the plant develops and matures (see tables 1, 4, and 7). In view of the results of the present investigation, Klason's hypothesis as to the mechanism involved in the phytochemical synthesis of lignin must be definitely rejected. It appears more probable that several intermediate substances are utilized by the plant in the synthesis of lignin. These substances do not contain the same percentage of methoxyl, and some may even be entirely free of this substituent. In the process of synthesis of lignin by the plant, these building units are combined in different proportions during the development of the plant. This would explain the fact that lignin isolated at successive stages of the development of the plant differs in its methoxyl content. Further research is required to establish the nature of the intermediate substances utilized by the plant in the synthesis of lignin.

SUMMARY AND CONCLUSIONS

The composition of the culms, sheaths, and leaves, chaff, rachises and branches of the panicle, and roots of the oat plant at successive stages of growth was studied with the object of determining the nature and percentage of the more important constituents, and thereby learning more about the formation of lignin by the growing plant and the possible mechanism involved in the process of lignification.

After an initial increase, the percentage of ash in the culms, sheaths, and leaves decreased, though irregularly, as the plant matured. The percentage of ash in the rachises and branches of the panicle showed a similar trend, while that of the chaff increased regularly. The percentage of ash in the roots was irregular. In the main, the absolute quantity of ash in the plant increased as the plant grew and matured.

The percentage of nitrogen (and crude protein) in the several parts of the oat plant studied, after an initial increase, decreased with maturity, with the exception of the chaff and the rachises and branches of the panicle, which showed a regular decrease. The absolute

quantity of nitrogen (and crude protein) increased rapidly during the early stages of the growth of the plant, and then decreased steadily, although not always regularly, as the plant developed further and matured.

The percentages, as well as the absolute quantities, of methoxyl in the original and in the extracted plant materials increased, in general, as the plant developed and matured.

In the earlier stages of growth of the plant, the weight of the various extractives increased rapidly, but in the later stage of its development it decreased somewhat. The only exception to this was the total 1-percent hydrochloric acid extractives, the figures for which show, in general, an upward trend.

After an initial increase, the percentage of uronic acids (as anhydrides) in the culms, sheaths and leaves, and the roots decreased slightly as the plant matured. In the chaff and in the rachises and branches of the panicle there was also a decrease, although an irregular one, as the plant grew older. The weight of the total uronic acids (as anhyrides) in the plant increased as the plant developed and grew to maturity.

The percentages of the total furfural and pentosans in the culms, sheaths, and leaves increased regularly and reached a maximum when the plant was 63 days old. Subsequently there was a decrease, but at or near maturity there was a slight increase. After a slight variation in the early development of the several parts of the plant studied, the percentage of furfural furnished by the uronic acids remained fairly constant. In the culms, sheaths, and leaves, the percentage of furfural yielded by the uronic acids, expressed as percentage of the total furfural, ranged from 23.4 in the early stages of growth to 6.8 at maturity. In the chaff and in the rachises and branches of the panicle, the percentage of furfural yielded by the uronic acids, expressed as percentage of the total furfural, ranged from about 6 in the early stages of growth to a little over 5 at maturity, while in the roots it ranged from about 8.5 to 6.25. The percentage of furfural yielded by the Cross and Bevan cellulose of the culms, sheaths, and leaves increased, though not regularly, as the plant grew older and matured. The percentage of furfural yielded by the pentoses of the polyuronides of the culms, sheaths, and leaves increased at first and then decreased irregularly. As the plant grew older, in each part except the roots more than 50 percent of the total furfural was furnished by the pentoses of the polyuronides. The weights of the total furfural-yielding substances and pentosans generally increased as the plant grew older and matured.

The percentages of Cross and Bevan cellulose, as well as the cellulose of the culms, sheaths, and leaves, and rachises and branches of the panicle, increased, but not regularly, as the plant grew older. In the roots the percentages of these constituents did not materially change. The percentage of xylan in the Cross and Bevan cellulose from the culms, sheaths, and leaves, rachises and branches of the panicle, and roots increased somewhat as the plant grew older, but the increase was neither consistent nor even. The weights of Cross and Bevan cellulose, xylan in the Cross and Bevan cellulose, and cellulose increased irregularly as the plant grew older.

The percentages of reducing and nonreducing sugars (calculated as glucose and sucrose, respectively) in the culms, sheaths, and leaves, chaff, and rachises and branches of the panicle increased in the early stages of growth and then decreased markedly as the plant grew older.

The percentage of lignin, as well as methoxyl in the lignin, in the culms, sheaths, and leaves, chaff, rachises and branches of the panicle, and roots generally increased as the plant developed and matured. The weight of total lignin also increased with the development and maturity of the plant.

In the fully mature plant, more than 80 percent of the total firmly bound methoxyl in the culms, sheaths, and leaves was found in the lignin. In the early stages of the development of the plant, the methoxyl not in the lignin, expressed as percentage of the total firmly bound methoxyl, was rather high, but the percentage of this decreased, in the main, as the plant grew older and matured. The lignin methoxyl, expressed as percentage of the total firmly bound methoxyl, in the roots ranged from 70.7 to 87.0.

The results obtained in this investigation indicate that the plant does not synthesize lignin from cellulose, pectin, or pentosans. The data are also not in harmony with Klason's hypothesis that coniferyl alcohol (or aldehyde) is the intermediate substance utilized by the plant in the synthesis of lignin. It is suggested that lignin is synthesized by the plant directly from either glucose or sucrose. Among the first steps in the synthesis of lignin is the production of a substance or substances having firmly bound (in etherlike combination) methoxyl groups, which may be formed in the course of splitting up carbohydrates by a process of hydrolysis, oxidation, reduction, and dehydration. The observed presence of large quantities of substances, presumably carbohydrates, containing firmly bound methoxyl groups and the gradual decrease of these substances with the increase in the content of lignin lends support to this hypothesis.

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A MOSAIC DISEASE OF CABBAGE[1]

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INTRODUCTION

For several years a mosaic disease has been observed on cabbage (*Brassica oleracea capitata* L.) in southeastern Wisconsin, but not until 1934 did it become widely prevalent. Preliminary investigations of the disease started in that year have already been reported by Blank (*4*).[3] The present writers took up the study in 1936 when the great destructiveness of the disease emphasized the need for more detailed information concerning the properties, vectors, and host range of the virus.

The masking of symptoms which may occur on cabbage has undoubtedly delayed recognition of the real severity of this disease. Its potential destructiveness was clearly emphasized in 1936 when widespread occurrence followed a heavy infestation of cabbage aphids which continued for the major part of the growing season. The yield from several thousand acres was reduced by approximately one-half, and the internal necrosis of heads which became evident at and following harvest resulted in still further losses.

The object of the present study was to record the symptoms of this disease on cabbage and other hosts, to determine the host range of the virus, to investigate the means of its transmission and overwintering, and to describe the virus as well as possible by a study of its properties. Preliminary reports have been published elsewhere (*21*, *44*).

PREVIOUS WORK ON VIRUS DISEASES OF CRUCIFERS

The first reports of a transmissible virus disease on cruciferous plants were made in 1921, when, as a result of independent investigations in two laboratories, the mosaic disease of turnip (*Brassica rapa* L.) was described. Gardner and Kendrick (*11*) at the Indiana Experiment Station transferred, by mechanical inoculation, the infectious entity from naturally occurring mosaic-diseased turnips to plants of the same species. They did not secure infection on radish (*Raphanus sativus* L.). Schultz (*28*), working concurrently at the Bureau of Plant Industry laboratories in Washington, D. C., had brought to his attention by W. A. Orton mosaic-diseased plants of turnip, Chinese cabbage (*Brassica pekinensis* (Lour.) Gagn.), and pot-herb mustard (*B. japonica* (Thunb.) Sieb.). He found that the infectious entity could be transmitted interchangeably between the three species

[1] Received for publication January 6, 1939. Cooperative investigations of the Wisconsin Agricultural Experiment Station and the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry U. S. Department of Agriculture.

[2] The writers express sincere appreciation to Eugene H. Herrling for making the photographs used in this paper. Valuable assistance was rendered by a grant from the Federal Works Progress Administration.

[3] Italic numbers in parentheses refer to Literature Cited, p. 390.

either by mechanical inoculation or by means of the aphid vector, *Myzus persicae* (Sulz.). A mosaic disease of Chinese cabbage has since been reported in many parts of the world (*1, 6, 8, 9, 15, 20, 23, 25, 35, 45*), and recently the disease on this plant has been described fully from California by Tompkins and Thomas (*42*). Equally widespread have been reports of turnip and rutabaga mosaic (*2, 5, 6, 10, 14, 13, 16, 26, 45*), the former having been described most completely in a recent paper by Tompkins (*40*). Other reports of mosaic on crucifers before 1930 were on *Raphanus sativus caudatus* (L.) Bailey from India (*19*), and on an unnamed species of *Raphanus* and on charlock (*Brassica arvensis* (L.) Ktze.) from Denmark (*13*). Between 1926 and 1929 a number of cruciferous hosts to the sugar-beet curly top virus were reported from the Pacific coast (*22, 29, 30*).

As early as 1910, Stewart (*34, p. 167*) observed in New York fields a necrotic flecking of the outer and inner head leaves of cabbage. He did not suggest a virus as the inducing agency, but it may well have been that his observations were concerned with the effects of cabbage mosaic. A chemical study of a mosaic disease of cabbage was reported by Jodidi et al. (*17*) in 1920, although these workers were unable to demonstrate that they were working with an infectious virus disease. In 1924 Spierenburg (*33*) in Germany noted internal flecking of cabbage which resembled the symptoms of cabbage mosaic. Following the preliminary report of cauliflower mosaic in New York by Thatcher (*36*), Clayton (*6*) reported extensive cross-inoculation studies with the virus from mosaic-infected rutabaga on Long Island. He failed to secure infection on cabbage although he transmitted the virus to its sister subspecies, cauliflower and Brussels sprouts (*Brassica oleracea botrytis* L. and *gemmifera* DC.) and to several other crucifers. The first authentic transfer of a mosaic virus to cabbage was reported in 1934 by Tompkins (*37*) in the transfer of cauliflower mosaic to that host. In 1935 Hoggan and Johnson (*16*), who obtained inoculum from a naturally infected turnip collected in southeastern Wisconsin by the senior author of this paper and from horse-radish collected in Illinois, secured positive infection of cabbage. It was obvious by this time that probably several mosaic viruses of crucifers occurred, and the last-mentioned authors were the first to make studies of the properties of this group (*16*). In this same year the first natural occurrence of mosaic on cabbage was described from southeastern Wisconsin by Blank (*4*). This was followed by reports from Oregon [4] and Washington.[5] A ringspot virus disease of cabbage was described from England by Smith (*32*) in 1935, but this disease appears to be distinct from mosaic and more likely is similar to the disease reported later from California by Tompkins et al. (*41*).

The full account of the cauliflower mosaic of the Pacific coast (*39*) shows it to be distinct from the disease described in this paper. Among the other cruciferous hosts upon which the natural occurrence of mosaic has been described recently are wild radish (*Raphanus raphanistrum* L.) (*43*), rape (*Brassica napus* L.) (*5, 18*), colza (*B. napus* L. *oleifera* DC.) (*18*), horseradish [6] (7), pe-tsai and Chinese mustard (*2*), honesty (*Lunaria annua* L.) (*24*), wallflower (*Cheiranthus cheiri* L.)

[4] McWHORTER, F. D. MOTTLING OR BREAKING IN DAME'S ROCKET IN OREGON. U. S. Bur. Plant Indus., Plant Dis. Reptr. 20: 199. 1936.

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[6] KADOW, K. J., and ANDERSON, H. W. BRITTLE ROOT OF HORSERADISH IN ILLINOIS. U. S. Bur. Plant Indus., Plant Dis. Reptr. 20: 288. 1936.

(*3, Rept. 21; 31*), dames rocket (*Hesperis matronalis* L.),[7] and stock (*Matthiola incana* R. Br.) (*12, 31, 38*). The mosaic disease of water cress (*Nasturtium officinale* R. Br.) (*3*), was shown to be due to a strain of cucumber virus 1. The identity of many of the other virus diseases just mentioned, however, remains in doubt because of the lack of

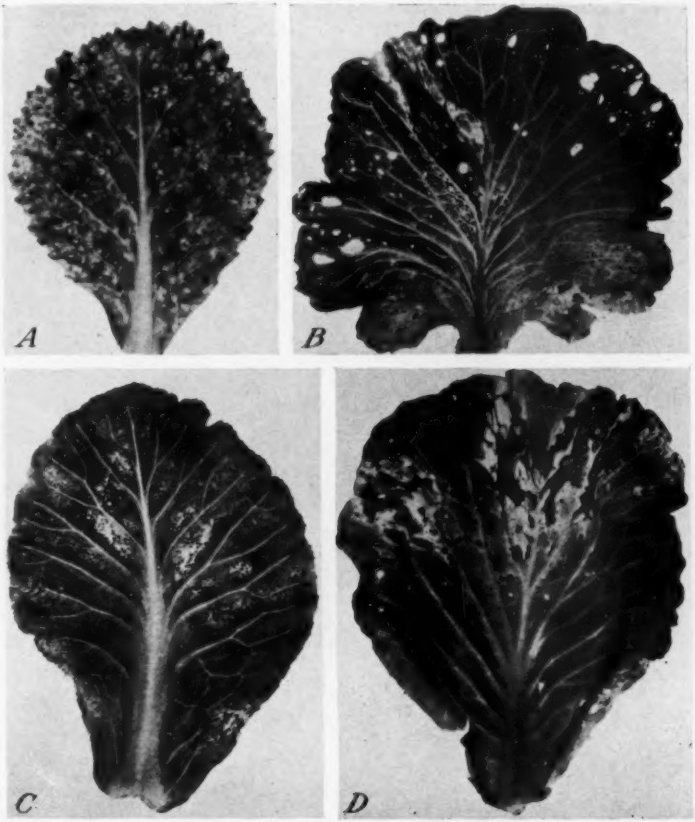

FIGURE 1.—*A*, Young infected leaf showing mottle and chlorosis; *B*, chlorosis followed by necrosis in a leaf in which infection has progressed for a longer interval; *C* and *D*, outer leaves of diseased mature cabbage showing chlorosis and bands of necrotic tissue. In *C* the necrosis is limited to an area extending from midrib to margin.

sufficient study of the virus concerned in each case. It is obvious that the time has come when the mere record of a mosaic disease of crucifers is of little value. A thorough analysis of symptomatology of the disease on various suscepts and a study of the properties of the virus are essential for distinction.

[7] See footnote 4.

SYMPTOMS OF MOSAIC DISEASE ON CABBAGE

The appearance of the mosaic disease as it occurs on cabbage in the greenhouse and under field conditions in southeastern Wisconsin will be considered first. Symptons do not develop in the inoculated leaves but rather in those which become infected later through systemic spread of the virus. The first symptom on young systemically invaded leaves is a slight yellowing with a clearing of the veins beginning usually at the distal portion of the leaf. This is followed by mottling and more distinct vein clearing (fig. 1, *A*). The symptoms which

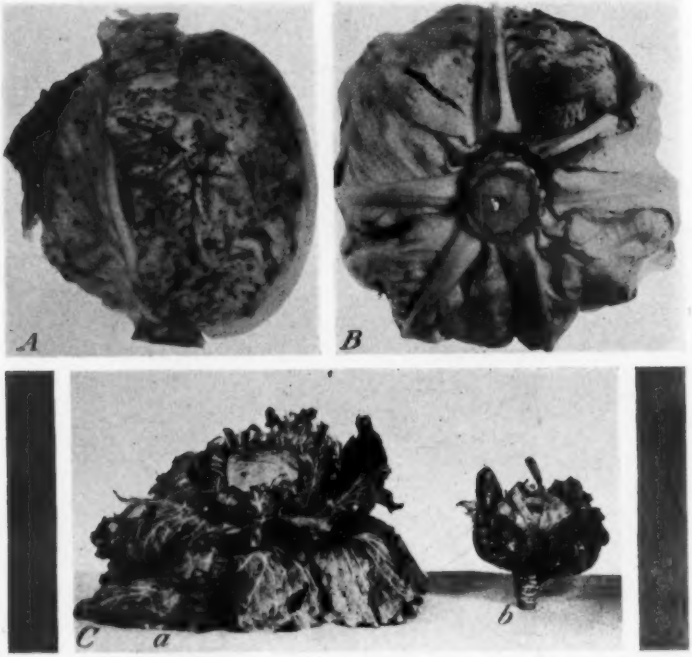

FIGURE 2.—*A*, Mature cabbage head infected with the virus showing internal necrosis of the parenchyma. *B*, Parting of leaves from core due to abscission-layer formation as a result of virus infection. *C*, *a*, uninoculated control; *b*, plant inoculated during the early part of the season, showing in comparison with the control the effect of progressive stunting and defoliation.

develop later vary considerably. They commonly consist of slight vein banding and savoying of the leaf lamina while the normal production of "leaf bloom" is retarded. The retardation of bloom produces a distinct off-type color in the bluer varieties, giving them a decidedly yellowish cast. As infected leaves become older, necrotic spots commonly occur on or along the veins and in the parenchyma (fig. 1, *B*). The spots on young inoculated plants are usually 1 to 3 mm. in diameter, sunken, and blue black in color. On the veinlets they may be linear, but on veins and midribs they are circular. The

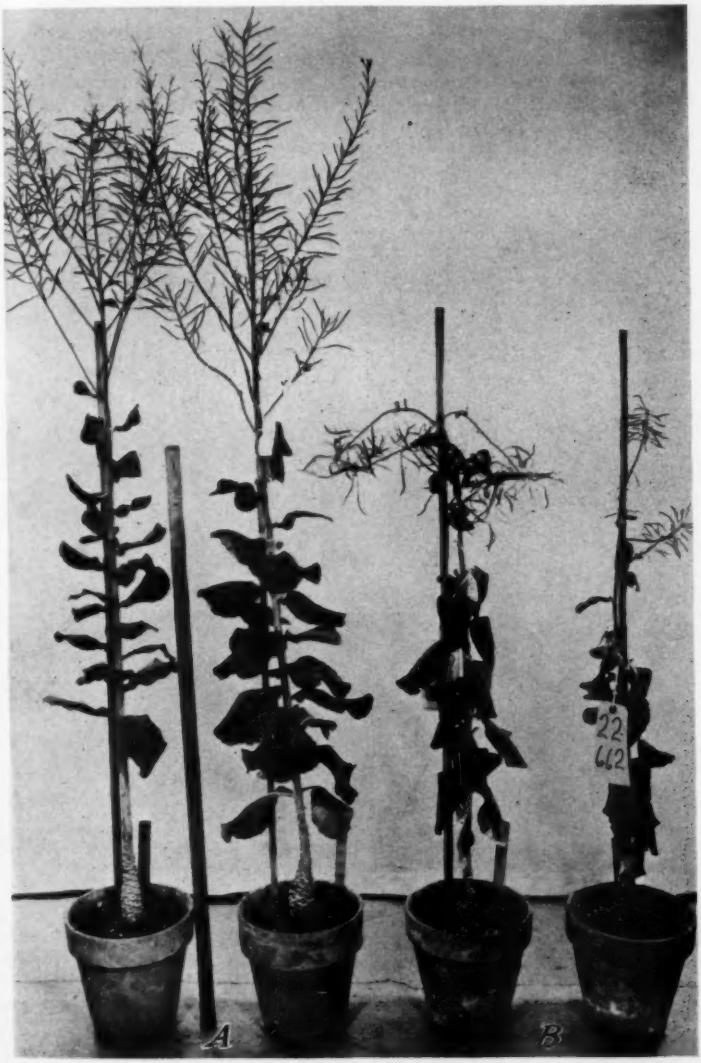

FIGURE 3.—*A*, Virus-free seed plants; *B*, advanced stage of virus symptoms on infected seed plants, consisting of declined vigor, defoliation, and impaired seed set.

savoying of the interveinal tissue, which is usually a deeper green than normal, is often followed by twisting or curling of the midrib.

In the field the symptoms may be masked very effectively, depending upon environment and possibly to some extent upon the variety. Warm midsummer weather is favorable to this disease, and in south-

eastern Wisconsin July and August are the months when it appears most conspicuously. Growth may be stunted without any sign of mottle or necrosis. Owing to leaf chlorosis and suppression of bloom, the color may appear yellower than normal. The outer leaves are inclined to be more erect and, being curved along the midrib, to clasp the head more tightly than is normal. The outer leaves show a variety of effects. Diffuse to prominent mottling may occur, sometimes followed by necrotic spots in the interveinal regions of the leaves.

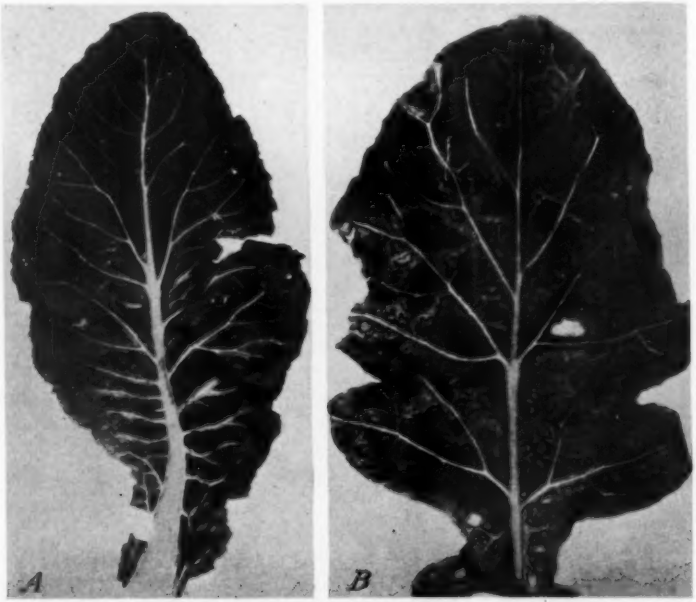

FIGURE 4.—*A*, Leaf of mature cauliflower infected with the virus, showing vein banding, mottle, chlorosis, distortion, and midrib twisting; *B*, chlorotic and necrotic mottle in the form of vein banding on sprouting broccoli.

Or, with little evidence of chlorosis, a necrosis may occur, either in spots or in continuous or interrupted bands which often extend to the edge of the leaf. These necrotic areas are brown, blue black, or purple, and as they grow older the tissue becomes dry and brittle (fig. 1, *C* and *D*). A common effect of systemic infection of cabbage is premature leaf drop. This may proceed whether or not other symptoms are conspicuous or even distinguishable. This condition results from premature formation of the abscission layer between the midrib or petiole and the main stem, and it is one of the most destructive phases of the disease (fig. 2, *C*).

Head necrosis also occurs (fig. 2, *A*, *B*), although it is not so frequent as the symptoms already described. It develops either as the head approaches maturity in the field or later in storage or transit. Sometimes it is confined to a few outer head leaves but it may be

scattered throughout the head. Commonly only a portion of a head leaf is affected from exterior to center. The necrosis is usually in the form of small, sunken, brown to black spots which are most numerous in the interveinal tissue where they may merge with one another to form larger spots. Black leaf speck may also result from suboxidation or low temperatures in storage, but this type is not associated with premature leaf drop. One common effect of the virus on the stored heads is the continuation of the untimely and premature formation of the abscission layer between petiole and main stem (fig. 2, *B*).

FIGURE 5.—*A*, Chinese cabbage inoculated with the cabbage mosaic virus, showing stunting, vein clearing, chlorosis, and leaf twisting, while the older leaves show necrosis; *B*, uninoculated control of the same age.

This effect may proceed up the entire length of the core, making the head worthless for market.

The masking of symptoms in cabbage plants is emphasized in holding them for seed production. It has been a common observation by the writers that normal healthy appearing plants selected for seed purposes often develop severe symptoms after the seed stem and branches have emerged in the greenhouse, or in the field the following season. Mottle of leaves, stems, and pods is common in such cases, followed by necrosis, drying, and premature dropping of the leaves. The vigor of such plants is often greatly reduced, and an increase in blasting of flower buds may occur (fig. 3). Necrosis of seed pods and impaired seed set have been often observed.

SYMPTOMS ON OTHER CRUCIFERS

The cabbage mosaic disease occurred in destructive form on cauliflower in southeastern Wisconsin in 1936, and the virus, when recovered, was found to be identical with that on cabbage in the same area. When young cauliflower plants are inoculated in the greenhouse the first symptoms are vein clearing, slight mottling, and yellowing of the younger leaves. The vein clearing gradually changes to vein banding, which consists of continuous, narrow, dark-green bands parallel with the midrib and veins but more conspicuous along the veins (fig. 4, *A*). Chlorotic bands along the veins may occur as well.

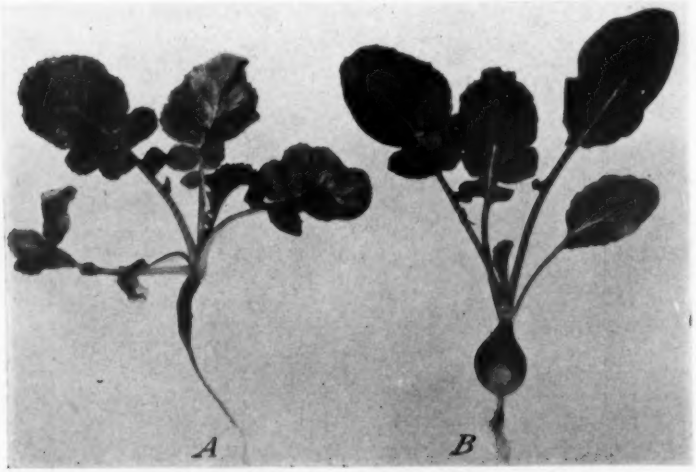

FIGURE 6.—*A*, Radish (Globe type) infected with the cabbage mosaic virus showing systemic infection, stunting, chlorosis, and retarded development of the fleshy hypocotyl; *B*, uninoculated control.

Mottle may consist of small spots or large areas. Necrosis appears later in the form of brown to blue-black linear lesions at the outer edges of the green or chlorotic bands along the veins. In the field vein banding and leaf distortion are particularly conspicuous as well as mottle and necrotic banding. Leaf distortion is much more conspicuous than on cabbage. The symptoms are often more severe on one side of the leaf than on the other and the resultant uneven growth causes much twisting of leaves and one-sided development of the plant as a whole.

In other subspecies of *Brassica oleracea*—kale, Brussels sprouts, kohlrabi, and sprouting broccoli—systemic vein clearing is again the first sign of infection, while conspicuous mottling, necrosis, leaf distortion, and general retardation of growth are conspicuous. Chlorotic mottle followed by necrotic bands parallel with the veins commonly develops on these hosts as on cauliflower (fig. 4, *B*).

Chinese cabbage is very susceptible to the cabbage virus. Vein clearing, chlorosis with some mottling, and necrosis are conspicuous. Leaf distortion is very common and pronounced general stunting of

the plant follows early infection under optimum environmental conditions (fig. 5).

Infected radish leaves develop vein clearing, mottle, slight chlorosis, and savoying. There is also some malformation and twisting. The plants are stunted and normal enlargement of the hypocotyl is retarded (fig. 6).

Symptoms on annual stock (*Matthiola incana* (L.) R. Br.) consist of vein clearing, chlorosis along the veins, mottling, and slight to severe stunting. Leaf malformation tends to be linear with some twisting and curling (fig. 7). Complete or sectional breaking of the flowers

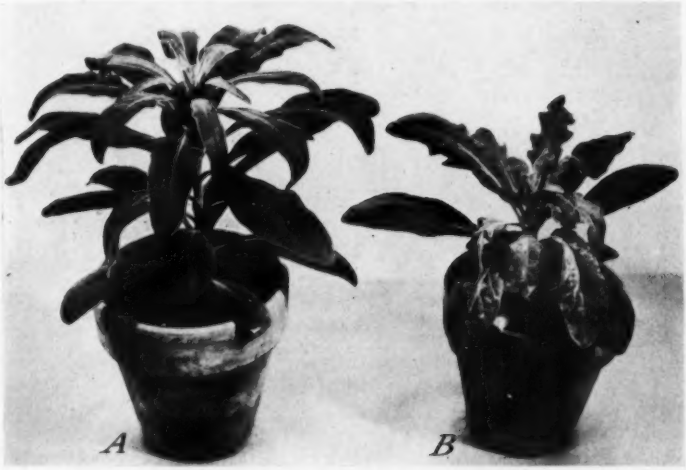

FIGURE 7.—*A*, Healthy *Matthiola incana* (annual stock), *B*, virus-infected plant showing stunting, vein clearing, and a limited amount of chlorosis.

in the form of streaks or bands results in distortion, with undersized and variegated petals (fig. 8).

Other cultivated crucifers found to be susceptible are listed later in the paper. The chief symptoms on all of these are varying degrees of systemic vein clearing, chlorosis, mottling, leaf distortion and stunting. The mottling is very conspicuous on dames violet (fig. 9).

TRANSMISSION STUDIES

METHODS AND MATERIALS

The virus used in these studies was obtained from cabbage grown in the yellows-resistance trial plot in Kenosha County, Wis., and has since been propagated on cabbage in the greenhouse at Madison, Wis. Frequent transfers were made to healthy cabbage to insure a constant supply of inoculum. The greenhouse temperature usually ranged from 22° to 25° C., and frequent fumigation for the control of insects was practiced. Artificial inoculations were made with extracted juice, and powdered carborundum was used regularly as an abrasive on cruciferous hosts (*27*), but was found to be unnecessary on many of the other susceptible hosts.

Healthy cabbage and turnip plants were employed for rearing colonies of the peach aphid (*Myzus persicae* (Sulz.)) and cabbage aphid (*Brevicoryne brassicae* (L.)). To increase vigor and to purify

FIGURE 8.—"Breaking" of flowers in the terminal racemes of *Matthiola incana* infected by the cabbage mosaic virus; petals undersized and variegated.

the cultures, new colonies were increased by transferring winged adults. Small numbers of viruliferous aphids were transferred directly to test plants with a camel's-hair brush, avoiding contact between the trans-

fer brush and the test plants. When a large number was to be transferred, a leaf with many aphids was detached and placed on a small piece of paper and the paper was allowed to rest on the leaf of the healthy test plant until the viruliferous aphids migrated to the plant. Reinoculations from test plants were made to tobacco and cabbage to determine the presence or absence of the virus. All plants tested for susceptibility to the virus were checked in this manner. Susceptible species were repeatedly tested and compared with uninoculated controls. Details of unusual methods are described later.

FIGURE 9.—Symptoms produced by the cabbage mosaic virus on leaves of dames violet: *A*, Leaf from uninoculated control; *B*, leaf from inoculated plant showing very conspicuous mottling.

CULTIVATED CRUCIFERS SUSCEPTIBLE TO THE VIRUS

All the cruciferous species tested were found capable of harboring the virus and of exhibiting symptoms when infected. The following list contains the forms tested; the symptoms on many of these have already been described.

Brassica oleracea viridis L. (kale—var. Dwarf Green Curled).
B. oleracea L. *gemmifera* DC. (Brussels sprouts—var. Long Island Improved).

B. oleracea capitata L. (cabbage—vars. Wisconsin Hollander, Wisconsin All Seasons, Jersey Queen, Marion Market, Golden Acre, Danish Ballhead, and Mammoth Rock Red).
B. oleracea botrytis L. (cauliflower—var. Snowball).
B. oleracea botrytis L. (broccoli—var. Green Sprouting).
B. oleracea gongylodes L. (kohlrabi—var. Early White Vienna).
B. napus L. (rape—var. Dwarf Essex).
B. campestris (L.) *napobrassica* DC. (rutabaga—vars. American Purple Top and White Russian.)
B. rapa L. (turnip—vars. Purple Top White Globe and Snowball).
B. juncea (L.) Coss. (leaf mustard—var. Tender Green).
B. pe-tsai Bailey (Chinese cabbage—var. Chihli).
B. alba (L.) Boiss (white mustard).
Cheiranthus allionii Hort. (Siberian wallflower).
Matthiola incana R. Br. (stock—var. Dwarf Ten Weeks).
Hesperis matronalis L. (dames violet).
Lepidium sativum L. (garden cress—var. Extra Curled).
Raphanus sativus L. (radish—vars. French Breakfast and Crimson Giant).

WILD CRUCIFEROUS HOSTS

All cruciferous weeds inoculated were found to be susceptible. Dwarfing and slight mottling with some vein clearing are the usual symptoms. In the case of shepherds-purse, wild peppergrass, and hoary alyssum the early symptoms on fairly old leaves appear in 10 to 15 days after inoculation as chlorosis along the leaf margins, slight savoying, and mottle, followed eventually by necrosis and death (fig. 10). Young leaves become dwarfed and curled, but they persist and give the plants a stunted appearance. This dwarfed, rosette appearance is typical of the naturally infected plants of these weeds found in the field. The seedstalks and pods from such plants are usually malformed.

The following species were tested. In each case the habit of the plant in the upper Mississippi Valley is indicated (A=annual; WA=winter annual; B=biennial; P=perennial).

Berteroa incana (L.) DC. (hoary alyssum) A or P.
Capsella bursa-pastoris (L.) Medic. (shepherds-purse) A or WA.
Neslia paniculata (L.) Desv. (ball mustard) A or WA.
Radicula palustris (L.) Moench. (marsh cress) A or B.
Sisymbrium officinale (L.) Scop. (hedge mustard) A or WA.
Sisymbrium altissimum L. (tumblemustard) A or WA.
Lepidium virginicum L. (wild peppergrass) A or B.
Lepidium sativum L. (peppergrass) A or B.
Thlaspi arvense L. (pennycress) A or WA.
Brassica arvensis (L.) Ktze. (charlock) A.
Brassica nigra (L.) Koch (black mustard) A.
Brassica juncea (L.) Coss. (Indian mustard) A.

NONCRUCIFEROUS HOSTS

In contrast to hosts that show a mottle and chlorosis when systemically infected with the cabbage mosaic virus, Swiss chard (*Beta vulgaris cicla* L.) and sugar beet (*B. vulgaris* L.) develop an erratic localized type of systemic lesions (fig. 11). The first symptoms exhibited are numerous small dark local spots. These increase in size, the center changing to a brick red, and on coalescence, necrotic areas develop in the parenchyma, giving the effect of vein banding. In the older infected leaves severe necrosis develops first at the distal portion and progresses toward the base. The tissues gradually become dry and brittle and the leaf dies.

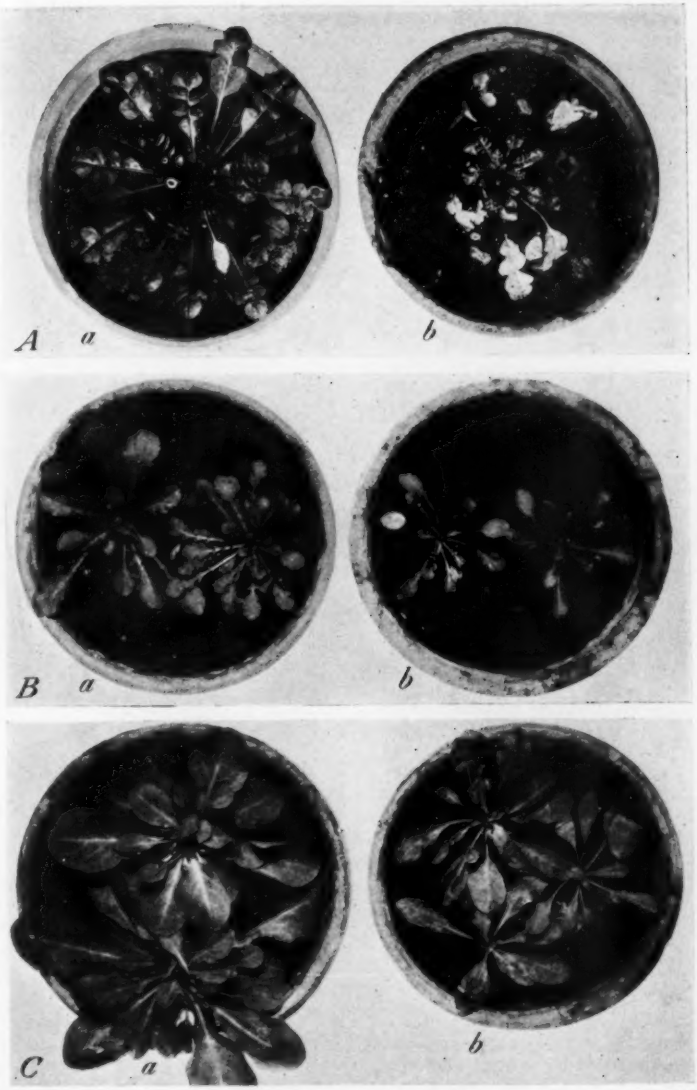

FIGURE 10.—Cruciferous weeds infected with the cabbage-mosaic virus: *A*, Shepherds-purse; *B*, wild peppergrass; *C*, hoary alyssum. *a*, Uninoculated controls; *b*, inoculated plants.

Systemic infection in spinach (*Spinacia oleracea* L. var. Bloomsdale) appears as conspicuous vein clearing, well-marked progressive chlorosis, and some necrosis. Considerable malformation of the leaves and stunting are very evident (fig. 12, *B*, *b*).

Infected larkspur (*Delphinium ajacis* L.) develops mottle with slight necrosis at the tips of the leaves. Later bleaching and vein banding of the leaves is evident. The older infected leaves become dry and brittle and drop. The infected plants are usually very much stunted. The flowers show breaking in the form of white streaks or flecks. Systemic infection in petunia (*Petunia hybrida* Vilm. var. Rose King)

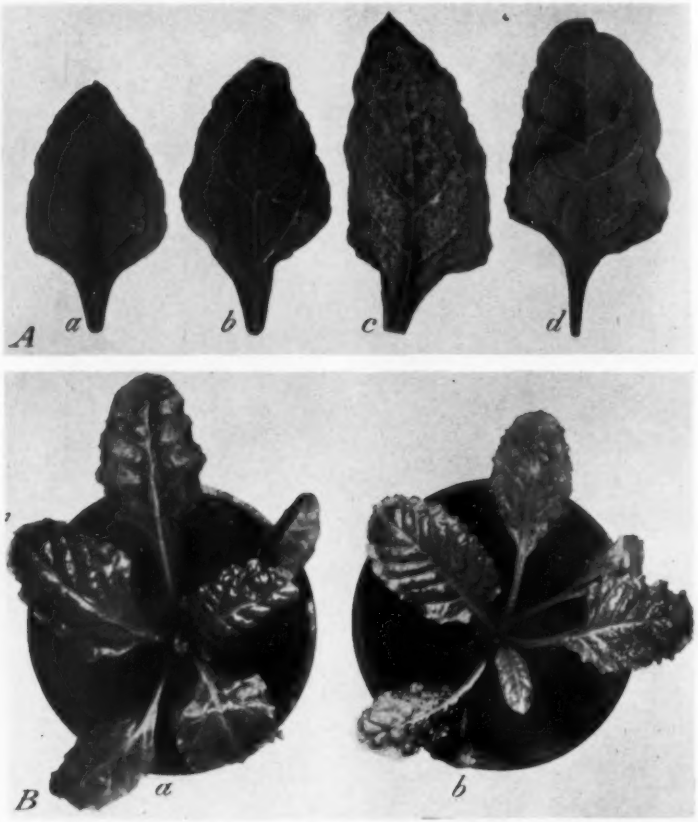

FIGURE 11.—*A*, Erratic type of systemic infection on leaves of sugar beet (*a*, *b*, *c*) infected with the cabbage-mosaic virus; uninoculated control (*d*). *B*, *b*, Infected plant of Swiss chard showing localized or spot type of systemic infection; *a*, uninoculated control.

results in a marked stunting, conspicuous mottle, and chlorosis without breaking of the flower (fig. 12, *A*). In calendula (*Calendula officinalis* L. var. Balls Orange) the first symptoms appear as clearing of the veins and mottle. This is followed by pronounced mottling and necrotic spotting. A twisting of the leaves and severe stunting are evident. Zinnia (*Zinnia elegans* Jacq. var. Orange and Gold) shows vein clearing followed by chlorosis and stunting.

REACTION OF SPECIES OF NICOTIANA

Infection of cruciferous and noncruciferous hosts described above was definitely systemic. In the species of *Nicotiana* tested infection was either localized, erratic (i. e., inoculation resulting in local lesions followed by systemic infection), or systemic (fig. 13). In tobacco (variety Connecticut Havana No. 38), *N. tabacum calyciflora* L., *N.*

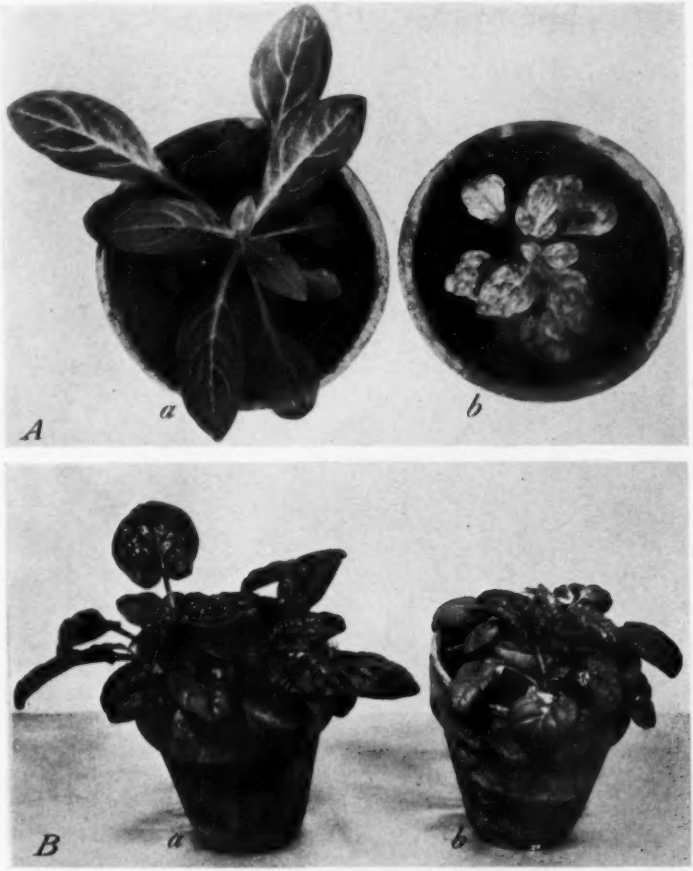

FIGURE 12.—*A*, *a*, Uninoculated petunia; *b*, petunia infected by the cabbage-mosaic virus, showing pronounced vein clearing, chlorosis, and stunting. *B*, *a*, Uninoculated spinach; *b*, virus-infected spinach plants showing stunting and conspicuous chlorosis.

sylvestris Speg., and in the F_1 hybrid of *N. tabacum*×*N. glutinosa*, small individual necrotic lesions appear on the inoculated leaves in from 3 to 4 days. The necrotic area enlarges rapidly up to 3 cm. or more in diameter, usually showing a brick-red center with concentric rings and a darker band at the edge. The older lesions become dry

and die out, and as they coalesce a large part of the leaf becomes involved. No systemic invasion occurs. *N. sylvestris* differed from the rest in that the local lesions did not appear on the inoculated leaves until 12 to 15 days after inoculation.

In *Nicotiana glutinosa* a faint halo type of chlorosis appears in from 12 to 15 days on systemically infected leaves, which is followed by a

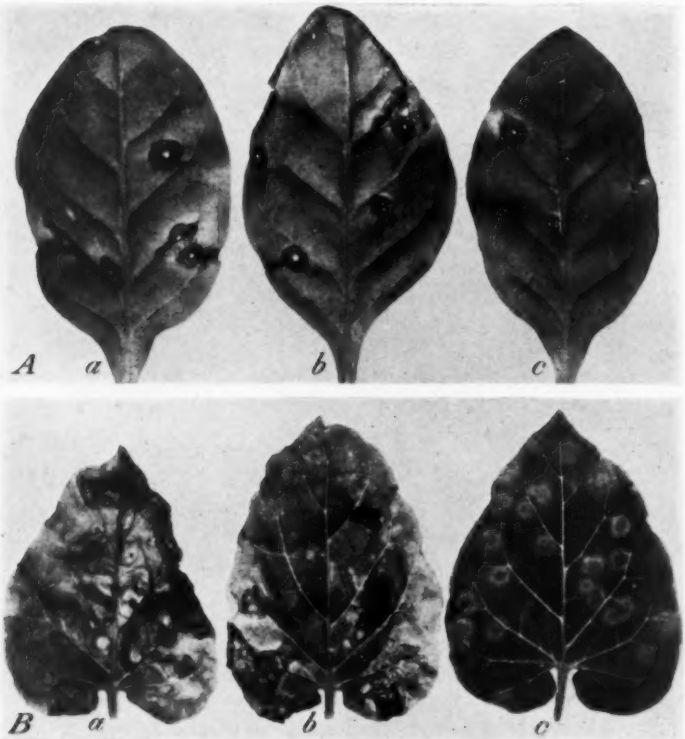

FIGURE 13.—*A*, *a-c*, Older local lesions on the F_1 hybrid of *Nicotiana tabacum*×*N. glutinosa* produced by transferring *Myzus persicae* from cabbage infected with the mosaic virus. *B*, Infected leaves of *N. glutinosa*: *a* and *b*, Older chlorotic lesions and necrosis; *c*, early halo type of chlorotic spots that form on systemically infected leaves to which the virus has spread from lower inoculated leaves.

more conspicuous yellowing with slight necrosis. The necrosis gradually involves the entire leaf but the virus is not fatal, for symptoms continue without preventing flowering and seed setting (fig. 13, *B*). In *N. rustica* L. no primary symptoms appear on the inoculated leaves. Secondary symptoms appear in about 15 days, consisting of irregular, diffused chlorosis and definitely marked progressive mottling of light and dark-green areas. Leaf distortion may follow in the form of one-sided infection and twisting (fig. 14, *A*, *a*, *b*).

In *Nicotiana repanda*, Sims primary local lesions appear in 3 to 4 days with dark centers and concentric rings. They enlarge and coalesce to form an irregular necrotic pattern, the inoculated leaves often becoming completely necrotic and dry. Early secondary symptoms consist of mild local chlorosis followed successively by mottling and necrosis. The plants usually are very much stunted. In *N. quadrivalvis*

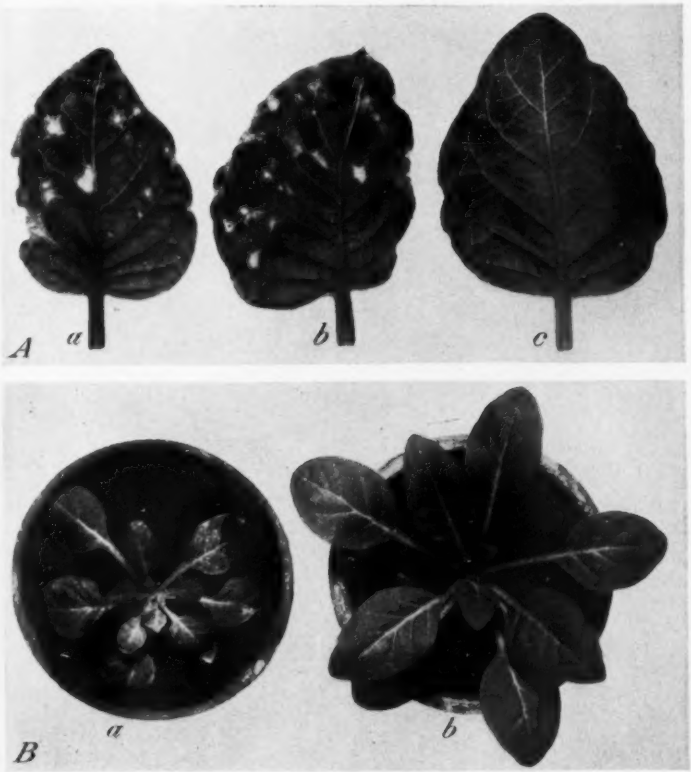

FIGURE 14.—*A*, Symptoms of systemic infection of *Nicotiana rustica* by the cabbage-mosaic virus are chloratic spotting and distortion (*a*, *b*); Healthy control (*c*). *B*, *a*, Stunting, vein clearing, chlorosis, and leaf malformation of *N. multivalvis* infected with the cabbage-mosaic virus; *b*, uninoculated control.

Pursh primary symptoms were not observed on the inoculated leaves. Vein clearing and yellowing of the younger leaves occurred in from 14 to 16 days, followed by stunting of the growing point. Leaf distortion in the form of twisting and narrowing appears with progressive chlorosis and savoying. No necrosis develops and the symptoms continue to appear until flowering. Chlorosis, vein clearing, and stunting of the younger leaves are evident on *N. multivalvis* Lindl. in about 15 days, with no primary symptoms on the inoculated leaves. The chlorotic patterns gradually become necrotic in older plants (fig. 14, *B*, *a*).

FIGURE 15.—Leaf symptoms produced by systemic infection of the cabbage-mosaic virus on *Nicotiana langsdorfii*, showing chlorotic mottling and distortion of the intercostal area.

Systemic infection appears in the form of conspicuous chlorosis on *N. langsdorfii* Schrank. Well-marked mottling of light- and dark-green areas with some leaf distortion follows (fig. 15). In *N. bigelovii* (Torr.)

S. Watts the virus produces pronounced, systemic infection in the form of conspicuous stunting and chlorosis 15 days after inoculation.

SPECIES NOT INFECTED BY THE VIRUS

Attempts to transmit the cabbage-mosaic virus to the following species were unsuccessful:

Yellow dock (*Rumex crispus* L.); buckwheat (*Fagopyrum esculentum* Moench), var. Silver Hull; pigweed (*Chenopodium album* L.); broadbean (*Vicia faba* L.); nasturtium (*Tropaeolum majus* L.); var. Golden Gleam; pansy (*Viola tricolor* L.), var. Black King; tomato (*Lycopersicum esculentum* Mill.), var. Globe; red currant tomato (*L. pimpinellifolium* Dunal); potato (*Solanum tuberosum* L.), vars. Irish Cobbler and Rural New Yorker; nightshade (*S. nigrum* L.); eggplant (*S. melongena* L.), var. Black Beauty; buffalo-bur (*S. rostratum* Dunal); *Nicotiana sanderae* Sander; *N. longiflora* Cav.; *N. nudicaulis* S. Wats.; snapdragon (*Antirrhinum orontium* L.), var. Rust Proof; muskmelon (*Cucumis melo* L.), var. Milwaukee Market; cucumber (*C. sativus* L.); var. Chicago Pickling; Watermelon (*Citrullus vulgaris* Schrad.), var. Stone Mountain; dandelion (*Taraxacum officinale* Weber); head lettuce (*Lactuca sativa* L. *capitata* Hort.), var. Iceberg; and China-aster (*Callistephus chinensis* (L.) Nees), var. Giant Blue (Wilt resistant).

INSECT TRANSMISSION

The species of insects found on cabbage in the field, viz, the cabbage aphid (*Brevicoryne brassicae*), green peach aphid (*Myzus persicae* Sulz.), and the imported cabbage worm (*Pieris rapae* L.) were studied as possible vectors. In view of possible variations in the concentration of the virus in leaves of different ages on the same plant, viruliferous aphids from leaves of corresponding ages were used in all transmission studies. Cabbage (var. Jersey Queen) and shepherds-purse were used as test plants. In tests with the cabbage aphid it was found that the feeding time required for nonviruliferous apterous forms to acquire the active principle was one-half hour and transmission of the virus was accomplished in a period of equal length. When the apterous nonviruliferous peach aphids were tested, the time to acquire the virus was slightly longer, although transmission of the virus to healthy test plants resulted after a feeding time of one-half hour. The short feeding period on both diseased and healthy test plant indicate quite definitely that a long incubation period of the virus within the two vectors does not take place. It was also found that viruliferous cabbage and peach aphids are capable of infecting four consecutive healthy test plants without intermittent feeding on the source of inoculum. A feeding period of 1 hour was allowed for each successive test plant. The possibility of reinfection of the aphids with accumulated virus from the test plant does not seem likely because of the short feeding period on consecutive test plants. The alate forms of both species were also shown to be capable of serving as vectors.

The cabbage worm was found to be effective in transmission of the cabbage mosaic virus in greenhouse tests. A small number, usually three, viruliferous cabbage worms, having previously fed on diseased plants for 24 hours, were transferred directly to the test plants and allowed to feed for as much as 4 hours and not more than 6 hours since longer feeding periods completely defoliated the test plants. In four series of 5 plants each, it was found that 16 of the 20 test plants produced typical symptoms.

TEMPERATURE RELATIONS OF THE DISEASE

The relation of temperature to the disease was studied under controlled conditions in the greenhouse. Healthy cabbage plants were artificially inoculated and an equal number (20) placed in each of a series of greenhouses which were kept as uniformly as possible at 16°, 20°, 24°, and 28° C., respectively. An equal number of checks was included. Two such experiments were conducted with similar results. At 16° a very slight mottling developed, visible with transmitted light only, and there was no vein clearing. Otherwise it was not possible to distinguish between inoculated and healthy plants. Slight symptoms occurred at 20° and a few plants showed vein clearing. At 24°, vein clearing and a distinct increase in mottling occurred with some necrosis on most plants. Pronounced symptoms occurred at 28°, although an increase in the chlorotic type of mottle developed. When diseased plants grown at the higher temperatures (24° or 28°) were removed to the cooler greenhouse (16°), new foliage of normal appearance developed. These series showed conclusively that the disease is expressed more rapidly and severely at high air temperatures, a result which is in full accord with the observations made upon the epidemiology of the disease in the field.

PROPERTIES OF THE VIRUS

In the study of the properties of the cabbage-mosaic virus, expressed juice of diseased cabbage plants was treated and then used to inoculate tobacco as the test plant. The average number of local lesions on one leaf of each of 10 plants in three trials each was used as the criterion for comparison (table 1). The longevity in vitro at about 20°–22° C. was found to be between 24 and 48 hours. The virus has an inactivation temperature at or near 55° for a 10-minute treatment and a dilution tolerance of about 1 to 1,000. The virus is readily inactivated by drying.

TABLE 1.—*Properties of the cabbage-mosaic virus as determined by local lesions formed on inoculation to tobacco*

[10 plants inoculated in each of 3 trials]

Longevity in vitro		Thermal death-point		Tolerance to dilution		Longevity in vitro		Thermal death point		Tolerance to dilution	
Period of exposure at 20°–22° C. (hours)	Average number of lesions on 30 leaves	Temperature of the 10-minute exposure	Average number of lesions on 30 leaves	Dilution	Average number of lesions on 30 leaves	Period of exposure at 20°–22° C. (hours)	Average number of lesions on 30 leaves	Temperature of the 10-minute exposure	Average number of lesions on 30 leaves	Dilution	Average number of lesions on 30 leaves
		°C.						°C.			
0	126	±20	130	0	141	48	9	54	3	1:1,000	1
12	92	52	121	1:10	81	72	0	55	0	1:2,000	0
24	24	53	12	1:100	11	96	0	56	0	1:3,000	0

In the study of virus diseases of cruciferous crops, reports of property studies have been given in only a few cases. A summary of the data reported are shown in table 2. Because of the uncertainty still existing concerning the number and identity of the viruses reported on cruciferous hosts, it seems well to indicate briefly the significant differ-

ence between the viruses mentioned in the table and the one described in this paper. The cabbage-mosaic virus reported herewith, when compared as to properties and host reaction with the mosaic on turnip of Hoggan and Johnson (*16*), appears to be similar if not identical. The cauliflower virus described by Tompkins (*39*) differs in the absence of infection on any species of *Nicotiana*, in the masking of symptoms at high temperatures (above 20° C.), and in each of the properties listed in table 2. The black-ring virus of cabbage differs from the cabbage mosaic virus in greater longevity in vitro, in a higher inactivation point, and in its low temperature optimum. The Chinese cabbage mosaic virus of Tompkins and Thomas (*42*) differs from the virus described herein in all of the properties studied and in the fact that it became systemic only in cruciferous hosts. The turnip mosaic virus of Tompkins (*40*) did not become systemic in cabbage.

TABLE 2.—*Comparison of properties of crucifer viruses as determined by different investigators*

Authority	Host	Virus disease	Longevity in vitro	Inactivation temperature (10 minutes)	Dilution tolerance
				°C.	
Hoggan and Johnson 1935 (*16*)	Turnip	Mosaic	24–48 hours at 22° C.	54	1–1,000
Tompkins 1937 (*39*)	Cauliflower	do	14–15 days at 22° C.	75	1–2,000
Tompkins et al. 1937 (*41*)	Cabbage	Black ring	72 hours at 22° C.	59	1–1,000
Tompkins and Thomas 1938 (*42*)	Chinese cabbage	Mosaic	72–96 hours at 22° C.	Between 73 and 75.	1–5,000
Tompkins 1938 (*40*)	Turnip	do	2–3 days at 22° C.	Between 60 and 63.	1–3,000
Larson and Walker	Cabbage	do	24–48 hours at 22° C.	55	1–1,000

RELATION OF THE VIRUS TO CABBAGE SEED

In 1936 mosaic was severe in the yellows-resistant cabbage plots in southeastern Wisconsin. There were some indications that infection occurred in the seedbed from which the plants were taken. This bed was in a secluded spot where crucifers had not been grown for many years. A large number of cabbage seed lots from a great many sources were included in this seedbed, and it is possible that the virus may have been introduced with one or more lots of seed.

During the winter of 1936–37 numerous diseased cabbage seed plants were grown in a cool greenhouse at 16°–20° C. for seed pod and seed formation. When extracted juice from young whole seed pods was used as inoculum all test plants developed virus symptoms. When only the young immature seeds (milk stage) were used as a source, the extract also proved to be infectious. Inoculations made with the extract from mature seed pods and with that from the mature seeds resulted in no infection of the test plants.

Mature seeds obtained from severely infected seed plants were used in seed-transmission trials in the greenhouse. A total of 5 plantings was made. All possible precautions were taken to prevent contamination from other sources. From a total of 1,764 plants, 26 were suspected of being diseased upon examination at the transplanting stage. These were reset for observation and it was found that 2 of the young plants showed definite mosaic symptoms and the juice extracted proved to be infectious. During the course of this experi-

ment between 3,000 and 4,000 plants were grown from seed from known healthy seed plants, in the same greenhouse, without any sign of disease at the transplanting stage.

While the data secured are not sufficient to serve as a basis for final deductions, two tentative conclusions seem to be clear. (1) The virus systemically invades the flower parts and the young pods and seeds, but if present in mature seeds it is there in such a state as not to be readily extracted. (2) The virus, if seed-transmitted, is not so perpetuated in a large percentage of cases even when seed is collected from very severely diseased pods. In spite of the very small number of cases of seed transmission, the growing of plants in crowded seedbeds and the effective means of spread by aphids combine to leave this means of virus introduction and dissemination a highly important one unless even rare seed transmission can be disproved with absolute certainty.

OVERWINTERING OF THE VIRUS

Since all cruciferous weeds found in Wisconsin that were tested proved to be susceptible, it was thought that the winter annuals and perennials that become infected in the latter part of the growing season as a result of migration of viruliferous cabbage or peach aphids might serve as an overwintering source of inoculum. A large number of cruciferous and noncruciferous weeds were collected in the early spring of 1937 before seedbed planting time. These weeds were growing on the borders of commercial cabbage and cauliflower fields that were heavily infected with the virus during the growing season of 1936. Many of the weeds collected were dwarfed and rosetted and their leaves were chlorotic and necrotic. Artificial and aphid (*M. persicae*) inoculations to young cabbage and shepherds-purse plants resulted in the recovery of the cabbage mosaic virus from only two cruciferous weeds, shepherds-purse and pennycress. None of the noncruciferous weeds yielded the virus. These two weeds are very common winter annuals in southern Wisconsin and may well become an important overwintering source of the virus or strains of the virus from which aphids may transmit to seedbeds and early transplanted fields in the spring.

RELATION OF CABBAGE VARIETIES TO THE DISEASE

In the course of field observations during the widespread occurrence of this disease in southeastern Wisconsin in 1935 and 1936 all the varieties exposed were infected. It was noted, however, that although general infection did occur some varieties were more severely damaged than others. Marion Market seemed to be most generally and severely affected, whereas Globe in adjacent plantings was less seriously damaged even though it sustained as high a percentage of infected plants. Wisconsin All Season seemed to produce quite successfully in spite of general virus infection.

Some preliminary results were secured at Madison, Wis., in 1936 in a comparison of inoculated and uninoculated plants of several standard varieties, all but one of which (Penn State Ballhead) had been selected for resistance to yellows (*Fusarium conglutinans* Wr.). One row of each variety was inoculated artificially with juice extracted from diseased plants, soon after the plants had recovered from trans-

planting. Thorough application of insecticides to control insect vectors was practiced throughout the growing season. At harvest weights of heads were taken from 10 inoculated and 10 uninoculated plants of each variety. The results are given in table 3.

TABLE 3.—*Comparative yields of healthy cabbage plants of different varieties and of plants inoculated with the cabbage-mosaic virus soon after transplanting*

Variety	Average weight of heads		Decrease of weight in diseased plants	
	Healthy	Diseased		
	Ounces	*Ounces*	*Ounces*	*Percent*
Penn State Ballhead	54.0	27.8	26.2	48.5
Marion Market	76.1	46.2	29.9	39.3
Wisconsin Ballhead	55.8	46.2	9.6	17.2
Wisconsin Hollander	49.2	41.3	7.9	16.1
Globe	63.0	53.3	9.7	15.4
Wisconsin All Seasons	71.5	63.7	7.8	10.9

These trials are too limited to furnish sufficient data upon which to base an evaluation of varieties. They do confirm, however, the field observation that Marion Market is more readily damaged than Globe and Wisconsin All Seasons. In this trial Penn State Ballhead appeared to be much more susceptible than the yellows-resistant varieties of the same type, Wisconsin Hollander and Wisconsin Ballhead.

DISCUSSION

It is obvious from the results of this investigation and that of other workers that a number of strains of virus affect the crucifers. Some of these appear to be confined to this family while others have a much wider host range. Some crucifers such as turnip and Chinese cabbage are very susceptible to all strains. On the other hand, some economic crucifers have a sufficiently differential reaction to aid somewhat in diagnosis.

The cabbage mosaic virus described in this paper seems to differ in several respects from crucifer viruses reported elsewhere. It is extremely virulent on cabbage in the Middle West, where its optimum temperature prevails at a time when the cabbage crop is usually just past the transplanting stage. Common cruciferous winter annuals in this area which become infected in late summer are capable of carrying the virus over winter readily. The cabbage aphid, which is commonly present, is an effective vector of the virus throughout the spring and summer. The customary procedure of growing plants in crowded seedbeds creates a situation especially favorable for extensive dissemination.

The recent sudden appearance of cabbage mosaic in destructive form cannot be explained with entire satisfaction. It has undoubtedly been present to a minor degree for many years. Symptoms are readily masked under a cool environment and many aspects of the foliage symptoms have undoubtedly been confused with those of other diseases.

Further study of the variation of this and other crucifer viruses is needed. In view of the findings in other virus groups it is reasonable to expect variations in respect to host range, properties, and virulence in this group. The extent of such variability will in some measure

determine the eventual severity of the disease and it will also have an important bearing on the progress that may be expected in the development of resistant varieties.

SUMMARY

The mosaic disease of cabbage discussed in this paper is one that has been extremely destructive to the crop in southeastern Wisconsin.

The symptoms brought about on cabbage and many cruciferous and noncruciferous hosts are described.

All cruciferous hosts, cultivated and wild, which have been tested were found to be susceptible.

Among noncruciferous hosts are three important crop plants, Swiss chard, sugar beet, and spinach, and three ornamentals, larkspur, petunia, and zinnia.

The reaction of several species of *Nicotiana* is given because of the importance of these facts in distinguishing the virus from others.

A list of plants that did not become infected upon inoculation is given.

Three insects found commonly in commercial cabbage fields, *Myzus persicae* (peach aphid), *Brevicoryne brassicae* (cabbage aphid) and *Pieris rapae* (cabbage worm) are vectors. *B. brassicae* picked up the virus from diseased cabbage plants in a feeding period of one-half hour and viruliferous aphids infected healthy test plants in a feeding time of one-half hour. *M. persicae* required 1 hour to secure the virus, although the feeding time for infecting healthy plants was the same as for the cabbage aphid. Both alate and apterous forms of the two species of aphids are vectors.

Environmental conditions play an important part in the destructiveness of the disease and expression of plant symptoms. The disease is most severe at temperatures of 24° to 28° C., within which range stunting and necrosis occur. New foliage appears symptomless when infected plants are held at 16° C. or lower.

The virus is transmitted mechanically with the aid of carborundum as an abrasive. It remains infectious in vitro for 24 to 48 hours at 20°–22° C. The tolerance to dilution is about 1 to 1,000; the virus is inactivated when held at about 55° C. for 10 minutes. Drying inactivates the virus.

The virus has been recovered from young seed pods and immature seeds of cabbage but not from mature pods or seeds. Seed-transmission has not been finally proved.

It has been definitely demonstrated that overwintering cruciferous weeds are an important source of inoculum in southeastern Wisconsin.

Indications from preliminary trials confirm field observation that certain cabbage varieties are more tolerant to the disease than others.

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VARIATION AND CORRELATION IN BUD MUTANTS OF THE MONTMORENCY CHERRY [1]

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INTRODUCTION

A bud mutant differs so radically in one or more respects from the parent form as to give rise to the question whether if propagated, it will be less stable in some, or even all, its characters, than similar but normal structures from the same parental source. Should the mutant show a tendency toward greater variation in one or more of its aspects, the further question arises as to whether this variation is also more heterogeneous. That is, does a lower degree of correlation obtain for this greater variability? If so, an unusual potentiality for further mutation might reasonably be expected.

MATERIALS AND METHODS

Intraclass correlations [2] were employed as a means of studying variation in mutants of the Montmorency cherry (*Prunus cerasus* L.)

The trees available for measurement were six in number, standing adjacent in a row, on the same type of soil, at the Graham Horticultural Experiment Station, Grand Rapids, Mich. They originated from a limb mutation on a tree in an orchard near South Haven, Mich. This limb, kept under observation for several years, in contrast to the rest of the tree, formed no fruit buds and was always barren. Buds were taken from it in the summer of 1925 and propagated in the nursery. The resultant trees were set in the orchard in 1929. Upon coming into bearing, they displayed the following characteristics, which remained the same in succeeding years and are so at the present time:

Tree 1—Barren, except two main limbs that have reverted to normal fruit-bud formation and productivity.

Tree 2—Wholly barren.

Tree 3—Barren, except one main limb with reduced but definite and regular fruitfulness.

Tree 4—Like tree 1, except that it has only a single fruitful limb.

Tree 5—Wholly barren.

Tree 6—Wholly normal in bearing.

[1] Received for publication October 14, 1938. Journal article No. 334 (n. s.) Michigan Agricultural Experiment Station.

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Of these six trees, four—Nos. 1, 4, 5, and 6—were used in the present study. These four provided two mixed specimens, one specimen entirely mutational, and one completely normal.

The character selected for measurement was leaf area (area of spur leaves), a character in which the range of variation is both wide and conspicuous.

The leaves were measured in situ. The length from base to apex, in millimeters, was converted into area by the use of a factor previously determined and established on the basis of printed and planed areas for excised leaves. Regularity of shape in the leaf of the Montmorency cherry permits such a procedure.

The data was gathered in midsummer, 1935. Measurement was restricted to nonbearing spur leaves of lateral branches on the wood of the year 1933. An additional set of measurements was secured from the 1934 wood on trees 5 and 6. Individual leaf areas were added to secure the total area per spur. Hence, in the logic and arrangement of intraclass correlation, each lateral branch, with its several spurs as fraternities, constitutes a general group.

PRESENTATION OF DATA

The results of the computations are shown in table 1.

TABLE 1.—*Intraclass coefficients for leaf areas*

Tree No.	Location of leaves	Pairings	Intraclass coefficients	z^1 values	σz^2	$z/\sigma z$
		Number				
1	Barren part	1,560	0.1534	0.51119	0.1858	2.8
	Fruitful part	1,008	.0794	.23910	.1827	1.3
4	Barren part	896	.1769	.49240	.1955	2.5
	Fruitful part	896	.1498	.42960	.1955	2.2
5	Barren tree	780	.2781	.59410	.1544	3.9
6	Fruitful tree	690	.1284	.30634	.1645	1.9
5	1934 wood	900	.1181	.41534	.2346	1.8
6	do	810	.0874	.33191	.2621	1.3

[1] $z=\frac{1}{2}\log_e\frac{1+(k-1)r}{1-r}$

[2] $\sigma_z=\sqrt{\frac{1}{2}\left(\frac{1}{n^1}+\frac{1}{n^2}\right)}$, with $n_1=n'-1$, $n_2=(k-1)n'$

The intraclass coefficients shown in table 1 are all positive. That for the barren part is apparently higher in each comparison. However, only three of the eight reach the level of probable significance. These three pertain to the barren parts of the trees. No one of the differences in the four comparisons of z is significant. The data, obviously inadequate as to number of observations, yield the suggestion, though not the full certainty, of real correlations in leaf area, those for the mutant forms being higher than those for the normal.

Incidentally, some information on variability as such, obtained by Fisher's method, is given in table 2.

TABLE 2.—*Mean squares for variance in leaf areas*

Tree No.	Location of leaves	Degrees of freedom	Sums of squares	Mean square	$\log_e$ (mean square)	Difference	$1/n$	Sums
1	Barren part	153	34,036.55	222.46	5.4048	0.0907	0.006536	0.014473
	Fruitful part	126	25,598.82	203.17	5.3141		.007937	
4	Barren part	112	35,639.40	318.21	5.7625	.1048	.008929	.017858
	Fruitful part	112	39,565.68	353.26	5.8673		.008929	
5	Barren Tree	130	20,708.28	159.29	5.0701	.1031	.007692	.016388
6	Fruitful tree	115	16,513.26	143.59	4.9670		.008696	
5	1934 wood	99	37,681.37	380.62	5.9419	.3557	.010101	.022446
6	do	81	21,602.37	266.70	5.5862		.012345	

Consider only the last comparison shown in table 2, in which the greatest difference exists. The difference of the logarithms is 0.3557. Therefore, z is 0.1779, or half this difference. The variance of z amounts to one-half the sum of the two reciprocals, and is 0.01122. The standard deviation of z is then the square root of this half sum, which is 0.1059.

The greater mean square for tree 5 is significant, since z is 1.68 times greater than its standard deviation. The differences shown in the other three comparisons do not reach the level of significance. Larger numbers of observations would be required thoroughly to test and establish their reality. The data suggest, although but slightly, greater variability in the mutants with respect to leaf area.

SUMMARY

A preliminary study has been made of variation and correlation in leaf area in bud mutants of the Montmorency cherry. The results furnish a mere indication of greater variability in the mutant than in the normal form and they suggest the probability that the variance in the mutant, as determined by intraclass correlation, is to a higher degree concomitant. The results give no apparent support to the hypothesis that a bud mutant may vary more heterogeneously than the normal form and therefore be especially predisposed to give rise to other mutations.

U. S. GOVERNMENT PRINTING OFFICE: 1939